Program Nr: 310 from the 1999 ASHG Annual Meeting

Evidence for a paternal effect on chromosome 7 in autistic disorder. *A. Ashley-Koch[1], C.M. Wolpert[1], M.M. Menold[1], L. Zaeem[1], S. Basu[1], S.L. Donnelly[1], S.A. Ravan[2], C.M. Powell[3], M.B. Qumsiyeh[1], A.S. Alysworth[3], J.M. Vance[1], J.G. Gilbert[1], H.H. Wright[2], R.K. Abramson[2], G.R. DeLong[1], M.L. Cuccaro[2], M.A. Pericak-Vance[1].* 1) Dept of Medicine, Duke Univ Med Ctr, Durham, NC; 2) W.S. Hall Psychiatric Inst, Univ of South Carolina, Columbia, SC; 3) Dept of Pediatrics, Univ of North Carolina, Chapel Hill, NC.

Autistic Disorder (AD) is a complex neuro-developmental genetic disorder with an estimated 2-10 loci underlying its genetic etiology. Several genome screens have been performed and have suggested at least one putative AD locus is located on 7q. We have previously reported a multiplex AD family in which three siblings and their mother are segregating a paracentric inversion on 7q (inv(7)(q22q31.2)). The two male siblings have AD; the female sibling exhibits expressive language disorder; the mother does not have AD. Haplotype data on the family suggests the inversion was inherited from the maternal grandfather. Based on these cummulative suggestive data, we performed a comprehensive analysis of our AD data set by genotyping 76 families for markers spanning a 30 cM region (D7S2527-D7S530-D7S640-D7S495-D7S684-D7S1824). Two-point analysis yielded a maximum parametric lod score of 1.12 at D7S1824, maximum heterogeneity lod score of 2.10 at D7S495, and maximum MLS of 0.97 at D7S2527 and D7S640. Multi-point MLS and NPL analysis resulted in peak scores of 1.55 at D7S2527 and 2.15 at D7S640, respectively. This linkage region overlaps the distal inversion breakpoint in our multiplex family. Examination of affected sibpairs revealed significant paternal (p=0.007), but not maternal IBD sharing (p=0.64) at D7S640. Using multiplex and singleton families, significant linkage disequilibrium was detected with paternal (p=0.02), but not maternal transmissions (p=0.15) at D7S1824. We also observed increased recombination in the region (D7S2527 to D7S1824) among AD families compared with non-AD families (p=0.01, sex-averaged and p<0.0001, sex-specific), suggesting this region exhibits increased instability in AD families. In summary, these results provide further evidence for the presence of a 7q AD locus, and suggest this locus may be paternally expressed.

Program Nr: 311 from the 1999 ASHG Annual Meeting

Evaluation of linkage disequilibrium between SNPs in a large outbred population. *S.H. Shaw[1], D.C. Hutchison[1], R. Saiz[1], L.E. Delisi[2], R. Sherrington[1]*. 1) Axys Pharmaceuticals, La Jolla, CA; 2) Department of Psychiatry, SUNY-Stony Brook, NY.

We have evaluated the extent of linkage disequilibrium in a large, outbred population by measuring linkage disequilibrium between marker alleles in a series of high density single nucleotide polymorphisms (SNPs) in an approximate 1Mb region of human chromosome 22q11. Nineteen SNPs were tested across this region with an average spacing of 57 kb (range=1.4-289 kb). These 19 SNPs were genotyped in a population consisting of 453 unrelated pedigrees that were largely collected in the U.S. and U.K. Haplotypes for all pedigrees were constructed using Genehunter and approximately 1400 unambiguous haplotypes from unrelated individuals in this population were evaluated for linkage disequilibrium between marker alleles. Every marker pair combination was tested (171 tests) and 2X2 contingency tables were constructed. Chi-square and corresponding p-values were computed for each pairwise test. In addition, several measures of linkage disequilibrium were calculated and compared to see how well they predicted distance. When comparing distance versus linkage disequilibrium (as measured by p-value), 14/16 (88%) of marker pairs less than 50 kb apart had p-values $< 5X10^{-8}$ (genome-wide significance for association studies), and all 16 marker pairs had p-values < 0.05. 9/18 (50%) of the marker pairs in the 50-100 kb range and 4/7 (57%) of the marker pairs in the 101-150 kb range also had p-values $< 5X10^{-8}$. It was noted that linkage disequilibrium was less robust between marker pairs with relatively large differences in allele frequencies. In addition, a region with no detectable linkage disequilibrium was observed between 2 SNPs (g04 and g05) which are only 60 kb apart. Interestingly, no SNP on either side of g04 and g05 were in linkage disequilibrium with markers across this interval, therefore creating a gap of detectable linkage disequilibrium in this region of chromosome 22. This finding most likely indicates a region of increased recombination located between these 2 markers. This data supports the use of high density SNP maps for disease linkage disequilibrium studies in outbred populations.

Program Nr: 312 from the 1999 ASHG Annual Meeting

Fast multipoint linkage calculation with Allegro. *D.F. Gudbjartsson[1,2], K. Jonasson[1], C.A. Kong[1,3].* 1) Decode Genetics Inc., Reykjavik, Iceland; 2) Institute of Statistics and Decision Sciences, Duke University, Durham, NC; 3) Department of Human Genetics, University of Chicago, Chicago, IL.

A new method for multipoint linkage calculation has been developed. The method achieves considerable speedup over previous methods, and allows larger families to be analysed. The method has been turned into a computer program, Allegro. Allegro has the same basic functionality as the well known Genehunter program, includes the features of Genehunter plus, and contains many improvements. Among the supported features are parametric and nonparametric LOD scores, nonparametric NPL scores, information, exact p-values, expected crossover rate, haplotyping and simulation. The program is simple to use and accepts the same data file format as Genehunter. It has been used extensively at Decode Genetics and typical speedup compared to Genehunter is 30-fold. On a computer with one Gb of memory the program can handle pedigrees with upto about 25 bits (for instance a family with 20 descendants and 15 founders), the corresponding maximum for Genehunter being about 20 bits.

Calculation of LOD scores involves three steps. Firstly, the determination of single point probabilities of individual inheritance vectors, secondly multipoint calculation, where the proximity of markers on the chromosome is taken into account, and thirdly the score calculation (which for parametric LOD scores involves peeling). The bulk of the calculation of Genehunter involves steps 1 and 3 and it is here that the improvements are largest. The key idea is to make use of tree traversal to avoid repeated calculation for similar inheritance vectors. This idea is utilized in the single point calculations, in the peeling, and in the calculation of nonparametric score statistics (for instance pairs score). Thus the time required for a typical Allegro run is governed by step 2, but even here there is significant speed-up. Genehunter employs so-called founder reduction to shorten inheritance vectors and speed calculation. Further bit reduction, founder couple reduction, has been developed in the new method, thereby gaining more speed.

Program Nr: 313 from the 1999 ASHG Annual Meeting

Lod score approach to direct identical-by-descent mapping based on genomic mismatch scanning. *R.S. Spielman*[1], *V.G. Cheung*[2], *W.J. Ewens*[3]. 1) Dept Genetics, Univ of Pennsylvania School of Medicine, Philadelphia, PA. 19104-6145; 2) Dept Pediatrics,Univ Pennsylvania School of Medicine, Philadelphia, PA; 3) Dept Biology, Univ Pennsylvania, Philadelphia, PA.

Direct identical-by-descent (IBD) mapping is a form of genetic analysis that combines two procedures: genomic mismatch scanning (GMS) to enrich for regions that are identical by descent (IBD) between two subjects affected with the same disease, and mapping on microarrays. A region that contains a disease-predisposing gene inherited from a common ancestor of some or all of the affected individuals is expected to show IBD that is increased over background IBD in subject pairs. We describe a method of statistical analysis to assess the strength of the evidence for increased IBD at each site tested in an ordered array representing positions along the chromosome(s).

In this analysis, we calculate a likelihood ratio for the data at each point in the microarray, representing sites along the chromosome. For each subject pair, the numerator is the likelihood of the observation (IBD or not) at that site, assuming that the probability of IBD is the observed proportion of IBD in the sample at that site. The denominator for each subject pair is the likelihood of the same observation, but given that the probability of IBD at the site is the proportion of IBD observed in that pair, over all sites tested. (This denominator corresponds to the background level of IBD.) We take the logarithm of the ratio and sum over all subject pairs to give a kind of lod score. In our examples, we illustrate the analysis with data from one chromosome, but the analysis can easily be extended to the entire genome.

The method is illustrated by re-analysis of the data of Cheung et al (Nat Genet 18:225, 1998) and with data for an artificial example of direct IBD mapping.

Program Nr: 314 from the 1999 ASHG Annual Meeting

Predictors of survival in neurofibromatosis 2. *M.E. Baser[1], J.M. Friedman[2], D.G.R. Evans[3].* 1) Los Angeles, USA; 2) Department of Medical Genetics, University of British Columbia, Vancouver, CAN; 3) Department of Medical Genetics, St. Mary's Hospital, Manchester, UK.

Preliminary data indicate that neurofibromatosis 2 (NF2) patients with severe phenotypes have increased mortality, but the natural history of the disease is poorly characterized. In cross-sectional studies, germ-line *NF2* mutation type is associated with age at onset of symptoms and number of intracranial meningiomas. We used the Cox proportional hazards model to assess clinical and molecular predictors of survival in the United Kingdom NF2 patients series (336 patients, 78 deceased, from 214 families). The patients were aged 4-86 yrs (median, 34 yrs), were diagnosed from 1945-1998 (median, 1990), and were followed for a median of 9 yrs after onset of symptoms. Of 144 patients from 77 families with identified germ-line *NF2* mutations, 71 had frameshift or nonsense mutations, 27 had splice-site mutations, 22 had missense mutations, 22 had large deletions, and 2 had in-frame deletions. The clinical variables considered were presence of vestibular schwannomas (VSs), non-VS cranial nerve tumors, spinal tumors, peripheral nerve tumors, lens opacities, and various symptoms associated with CNS tumors; number of intracranial meningiomas; ages at onset of symptoms and at diagnosis; calendar year of diagnosis; and proband status. Four clinical variables were independent predictors of survival. The relative risk of death decreased 3.61-fold per decade increase in age at diagnosis ($p < .001$) and 1.79-fold per decade increase in calendar year of diagnosis ($p < .001$); increased 1.76-fold per intracranial meningioma ($p = .002$); and was 2.23-fold higher in probands ($p = .007$). There was a possible interaction between age at diagnosis and number of meningiomas ($p = .055$). Germ-line *NF2* mutation type did not predict survival in these data. These results indicate that readily-characterized clinical variables are associated with survival in NF2.

Program Nr: 315 from the 1999 ASHG Annual Meeting

Lifetime breast cancer risk associated with BRCA 1 and BRCA 2 mutations among patients of Ashkenazi Jewish origin. *C.B Begg[1], J.M Satagopan[1], M. Robson[2], K. Offit[2].* 1) Epidemiology and Biostatistics, Mem Sloan-Kettering Canc Ctr, New York, NY; 2) Clinical Genetics Service, Mem Sloan-Kettering Canc Ctr, New York, NY 10021.

BACKGROUND: A very high lifetime penetrance for breast cancer in carriers of BRCA 1 and BRCA 2 mutations is provided by several family-based studies. These studies evaluate multiple affected family members. Penetrance estimate can also be obtained directly by using a consecutive series of incident breast cancer cases seen in one large hospital, or from several institutions. A large population-based control series can be used to provide age-specific prevalence of the mutation, relative risk estimates, and the lifetime penetrance. METHODS: We evaluated 298 women self-identified as being Jewish who were treated for early stage breast cancer at Memorial Sloan-Kettering Cancer Center between 1980 and 1990. Fodor et. al. (1998) published cancer risk in a similar hospital based case-selection of 268 breast cancer patients treated between 1986 and 1995 at Mount Sinai Medical Center, New York. These data were combined to give an aggregated case series of 566 patients. Control data were obtained from a large population series of 3440 Jewish women from the Washington D.C area evaluated by Struewing et. al. (1997). Cases and controls were genotyped for the following founder mutations: 185delAG, 5382insC, and 617delT. RESULTS: We estimated the relative risk of breast cancer to be 10.8 in women under age 40, 5.5 for women in the age group 40-49, and 4.4 in women above age 50. Mutation prevalence are reported in Struewing et. al. (1997). Assuming that the population incidence of breast cancer in Jewish women are similar to the population rates in American women (as in the SEER database), and based on the above age-specific relative risk estimates and the published prevalence estimates, the penetrance of breast cancer at age 70 among founder mutation carriers is 29%. CONCLUSION: The lifetime risk of breast cancer among mutation carriers derived using a population-based approach as above is substantially lower than the risk reported by high-risk family studies.

Program Nr: 316 from the 1999 ASHG Annual Meeting

Tissue microarray FISH and digital imaging: Towards automated analysis of thousands of tumors with thousands of probes. *L. Bubendorf[1], J. Kononen[1], M. Barlund[1], A. Kallioniemi[1], A. Grigorian[2], G. Sauter[3], E.R. Dougherty[2], O.P. Kallioniemi[1]*. 1) Cancer Genetics Branch, NHGRI/NIH, Bethesda, MD; 2) Texas A&M University; 3) University of Basel, Switzerland.

Using our recently developed tissue microarray technology (Kononen et al., Nat. Med. 4:844-7, 1998), up to 1000 tissue specimens can be analyzed by fluorescence in situ hybridization (FISH) in a single experiment. Furthermore, it is also possible to produce tens of thousands of replicate tissue microarray sections from the same clinical material, providing templates for either genome-scale search for genetic alterations, or focussed high-resolution surveys of specific chromosomal sites. As an example of the latter approach, we studied 17q23 amplicons in breast cancer by hybridizing BAC probes from a 3 Mb genomic contig on a breast cancer tissue microarray (n=668). Almost 5000 FISH results were generated, resulting in a rapid definition of the critical region of this novel amplification, present in 8.8% of primary breast cancers, with a significant association with poor prognosis (p=0.002). To further explore the potential of tissue microarray FISH and to develop automated image analysis, we applied a new confocal digital imaging system (CARV, Atto Instuments, Rockville, MD) to acquire images of the arrayed tissue specimens (X-Y scanning) at different focal planes (Z-axis scanning). Relative copy numbers of test vs. reference probes were calculated over a defined field of view in each specimen at different focal planes (along the Z-axis) with a new 3D spot counting algorithm. Taken together, tissue microarray FISH, and automated digital imaging will enable genome-scale analysis of genetic alterations in thousands of clinical specimens.

Program Nr: 317 from the 1999 ASHG Annual Meeting

Association of the I1307K APC mutation with hereditary and sporadic breast/ovarian cancer. *E. Dagan[1,2], R. Gershoni-Baruch[1,2], Y. Patael[3], R. Bruchim-Bar-Sade[3], E. Friedman[3].* 1) Human Genetics, Rambam Medical Ctr, Haifa, Israel; 2) Bruce Rappoport Faculty of Medicine, Technion-Institute of Technology, Haifa, Israel; 3) Susanne Levy Gertner Oncogenetics Unit, Chaim Sheba Medical Ctr, TLV, Israel.

Females with BRCA mutations have a 40-60% lifetime risk of developing breast and/or ovarian cancer. The incomplete penetrance of BRCA genes mutations, suggests that other factors determine the phenotypic expression of mutant BRCA alleles. In Ashkenazi Jews three predominant founder mutations in BRCA1/2 (185delAG, 5382insC and 6174delT) were described. Lately, it has been suggested that the I1307K APC polymorphism associated with increased colon cancer risk, occurs in 6-7% of the Ashkenazi population and may act as a low penetrance gene/modifier in sporadic or hereditary breast cancer. To test this notion, BRCA carriers with either breast and/or ovarian cancer (n=143); or are asymptomatic (n=53), were tested with respect to being co-carriers of the I1307K mutation. Similarly, the prevalence of I1307K mutation was determined in non BRCA carrier, Ashkenazi breast/ovarian cancer patients (n=382) and in ethnically matched healthy controls (n=418). Twenty three of the controls (5.5%) and 17 of sporadic breast/ovarian cancer patients (4.6%) carried the I1307K mutation (p=ns). Of 196 BRCA mutation carriers, 17 carried the I1307K mutation, a rate (8.7%) although higher, not significantly different than in controls. The APC mutation was equally prevalent in symptomatic BRCA carriers (10/143; 7%), sporadic breast/ovarian cancer patients (17/382; 4.6%) and asymptomatic BRCA carriers (7/53; 13.2%). The co-mutation carrier status is similar in women diagnosed with breast cancer before (4/55; 7.3%) and after (5/59; 8.5%) 42 years. The I1307K mutation was equally prevalent among patients with inherited (9/114; 7.9%) sporadic (16/357; 4.9%) breast cancer, and controls (23/418; 5.5%). Co-carriers of the Jewish mutations in BRCA and APC, are not at higher risk for breast/ovarian cancer. The notion that the I1307K polymorphism may act as a low penetrance gene/modifier or is rather associated with increased breast cancer risk is weakened.

Program Nr: 318 from the 1999 ASHG Annual Meeting

Mutations in sporadic tumors in the BRCA1-interacting protein CtIP and the evaluation of its association with the ubiquituous co-repressor CtBP in mammalian cells. *F. Du[1], B. Weber[2], R. Baer[3], A. Bowcock[1]*. 1) Div.Hum.Genet.,Dept.Genet., Washington Univ. Med. Ctr., St. Louis, MO; 2) Dept. Genet. and Med., Univ.Pennsylvania, Phila,PA; 3) Dept.Micro., UT SW Med Ctr., Dallas,TX.

The protein encoded by CtIP interacts in vivo with the BRCT domains of BRCA1.This interaction is abolished by tumor-associated mis-sense mutations in this region. CtIP was originally identified on the basis of its association with the CtBP transcriptional co-repressor. To further investigate the role of CtIP in sporadic and hereditary cancer, we first determined its genomic structure by direct sequencing of bacterial artificial chromosome DNA and determine that CtIP is encoded by 18 exons spanning ~ 100kb. We then used DHPLC to screen for genetic alternations in coding sequence in a panel of genomic DNAs/cDNAs from patients/tumors with sporadic breast/ovarian/uterine cancer. One endometrial adenocarcinoma harbored a serine deletion at codon 439, but also retained the wt allele. The BT474 cell line harbored a Glu(510)Gln alteration and one high risk breast cancer patient harbored an Asp(540)His change. CtIP also harbors a polymorphism (Arg589His). The His allele is seen in 2% Caucasians. In order to confirm the interaction between CtIP and CtBP in-vivo, we performed a mammalian two-hybrid assay. A Gal4p-responsive reporter (luciferase) gene was transfected into mammalian cells along with an expression plasmid encoding either full-length CtIP fused to the transactivation domain of VP16 or full-length CtBP fused to the DNA-binding domain of GAL4p. Although neither fusion protein activated the reporter construct on its own, we observed a significant increase in luciferase activity when cells were co-transfected with both expression plasmids. This confirms that CtIP interacts in-vivo with the ubiquituous co-repressor, CtBP. The interaction is mediated by the "PLDLS" motif within exon 10. Further mammalian two-hybrid assays indicate that exon 10 of CtIP alone is insufficient for efficient interaction between the two proteins. BRCA1 has recently been implicated in a variety of cellular processes including DNA repair, transcription and replication. The CtIP/CtBP complex may mediate some of these.

Program Nr: 319 from the 1999 ASHG Annual Meeting

Loss of function mutations in the gene encoding peroxisome proliferator-activated receptor gamma (PPARgamma) in colorectal carcinomas. *C. Eng[1], P. Sarraf[2], W.M. Smith[1], E. Mueller[2], J.B. Kum[1,2], H.M. Wright[2], L.A. Aaltonen[3], A. de la Chapelle[1], B.M. Spiegelman[2].* 1) Human Cancer Genetics Program, Ohio State Univ Comp Cancer Ctr, Columbus, OH; 2) Cancer Biology, Dana-Farber Cancer Inst, Boston, MA; 3) Medical Genetics, University of Helsinki, Helsinki, Finland.

The lifetime risk of developing colorectal carcinoma is approximately 5%, of which the majority are sporadic. Stepwise accumulation of somatic genetic alterations contributes to the development from normal colonic mucosa to adenomatous polyp with subsequent progression to dysplastic polyp and invasive carcinoma. Human colon cancers express PPARgamma, a member of the nuclear receptor superfamily involved in fat cell differentiation and glucose homeostasis. Ligand activation of this receptor causes most, but not all, colon cancer lines to undergo a differentiative response and to reverse the malignant phenotype. Paradoxically, however, ligand activation in min mice, the murine model of human familial adenomatous polyposis, caused increased polyposis. Since 1.6 million patients take the anti-diabetic drugs that function as PPARgamma ligands, determination of the role of this receptor in human colorectal tumor biology is critical. Among 55 sporadic colorectal carcinomas, 12 (22%) somatic mutations were found. Of the 35 cancers that were microsatellite stable (MSI-), 2 (6%) were found to have somatic mutations, Q286P and K319X, both within the ligand binding domain. Of the 20 MSI+ tumors, 10 (50%) carried somatic mutations, 472delA, R288H and IVS2+7-+15delA (N=8). The IVS2+7-+15delA mutation is of unclear significance. 472delA results in deletion of the entire ligand binding domain and hence, is presumed to be inactive. Q286P and K319X retain a total or partial ligand binding domain, but lose their ability to activate transcription through failure to bind to ligands. R288H showed a normal response to synthetic ligands but greatly decreased transcription and binding when exposed to natural ligands, Prostaglandin J2, 9(S)-HODE or 15(S)-HETE. These data in humans indicate that colon cancer in humans is associated with loss of function mutations in the gene encoding PPARgamma.

Program Nr: 320 from the 1999 ASHG Annual Meeting

Functional analyses of *PPP2R1B* mutations in small-cell lung carcinoma. *E.D. Esplin, J.L. Li, S.W. Long, P.M. Ramos, D. Stickens, S.D. Thomas, S.S. Wang, G.A. Evans.* McDermott Center, UT Southwestern Medical Center, Dallas, TX.

PP2A is a serine/threonine protein phosphatase involved in the control of cellular growth including down-regulation of the mitogen-activated protein kinase (MAPK) cascade. It plays a critical role in oncogenesis as a target for chemical tumor promoters, SV40 small t antigen, and the HOX11 oncogene. PP2A is composed of a catalytic C subunit, regulatory B subunit, and structural/regulatory A subunit. Several isoforms of each subunit exist, leading to a variety of structural forms of the PP2A holoenzyme. We recently identified mutations in the *PPP2R1B* gene, encoding the b isoform of the A subunit of PP2A, in lung tumors, colon tumors and lung tumor-derived cell lines. To characterize the affects of these mutations on protein function and tumorigenesis, we examined protein-protein interactions between the normal and mutated subunits of the holoenzyme. Both alleles of *PPP2R1B* in small-cell lung carcinoma (SCLC) cell line H1450 carry mutations in the C-terminus. Each of these mutations results in A subunits with decreased affinity for the C subunit. To test whether these mutations affect enzyme activity, we analyzed the total phosphatase activity of H1450 cell lysates using phosphorylated myelin basic protein. We found a decrease in total H1450 phosphatase activity, suggesting PP2A activity is decreased toward some substrates as a result of *PPP2R1B* mutations. Known substrates of PP2A include MAP/ERK kinase (MEK), a key component of the MAPK cell growth and differentiation pathway. We examined the phosphorylation state of MEK 1 and MEK 2 in cells carrying *PPP2R1B* mutations. Two of these cell lines showed the presence of hyperphosphorylated protein. These results suggest mutations in the *PPP2R1B* gene cripple the ability of PP2A to control cell growth through the MAPK cascade. To further assess the tumor suppressor activity of PP2A, we developed a model of lung tumorigenesis using nude mice and the SCLC cell line H1450. The expression of *PPP2R1B* by transfection into H1450 cells provides a useful assay for *in vivo* tumorigenesis mediated by mutations in *PPP2R1B.*

Program Nr: 321 from the 1999 ASHG Annual Meeting

Molecular genetic testing of a population based sample of women with breast cancer aged 30 years or less. *G. Evans[1], F. Lalloo[1], E. Redmond[1], C-L. Wu[1], B. Bulman[3], A. Moran[2], L. O'Dair[1], J. Varley[3], C. Mathew[4].* 1) University Department of Medical Genetics and Regional Genetics Service, St Mary's Hospital, Manchester, UK; 2) Department of Epidemiology, Christie NHS Trust, Manchester, UK; 3) Paterson Institute of Cancer Research, Christie NHS Trust, Manchester, UK; 4) Department of Molecular Genetics, Guys Hospital, London,UK.

Women diagnosed as having breast cancer at age 30 years or under on the Manchester Cancer Registry since 1980 have been contacted to obtain details of their family history and a blood sample. These have been obtained on 72 women and screening of the BRCA1 and BRCA2 genes have been undertaken. In addition families fulfilling criteria for Li Fraumeni Syndrome (LFS) or Li Fraumeni Like (LFL) have been analysed for mutations in TP53. Forty-four women had an isolated case of breast cancer without no first or second degree relative with breast or ovarian cancer under 60 and only one of these had a previously unreported missense mutation in BRCA1. Two women had an aggregation of tumours consistent with LFS and in one a mutation was identified in TP53. Of 2 further women fulfilling LFL criteria, one had a mutation in TP53. The TP53 negative woman also screened negative for mutations in BRCA1 and BRCA2. This left 24 women with a further history of breast or ovarian cancer in first or second degree relatives diagnosed under the age of 60. To date, 10 of these women have had a mutation identified in BRCA1 and one in BRCA2. TP53 testing of all women without mutations in BRCA1/2 will also be completed. These results show that breast cancer diagnosed at the age of 30 or younger in the context of a further family history is a strong predictor for the presence of inherited mutations in BRCA1, BRCA2, or TP53. Of the 12 families with mutations in BRCA1/2, there were 19 breast cancers (mean age 42) in first and second degree relatives and 7 ovarian cancers. The pattern of cancers in these families suggest that the mutations found are associated with a high lifetime risk of developing breast and/or ovarian cancer contrary to the reports of other population studies.

Program Nr: 322 from the 1999 ASHG Annual Meeting

Germline BRCA mutation is an adverse prognostic factor in Ashkenazi Jewish women with breast cancer: Survival disadvantage depends on nodal status. *W.D. Foulkes[1], N. Wong[1], J-S. Brunet[3], M. Trudel[1], A-J. Paradis[1], G. Kuperstein[3], P.O. Chappuis[1], L. Kapusta[2], J. Slingerland[2], S.A Narod[3], L.R Bégin[1].* 1) Depts Medicine, Pathology, Surgery and CPRU, Montreal General and Jewish General Hospitals, McGill University, Montreal, QC, Canada; 2) TSRCC, University of Toronto, Toronto, ON, Canada; 3) Women's College Hospital, Toronto, ON, Canada.

Germline mutations in BRCA genes account for >10% of breast cancer (BC)diagnosed under 65 yrs of age in Ashkenazi Jewish (AJ) women. The clinicopathologic features of these cancers are known but the prognosis remains uncertain. We used a historical cohort approach to answer this question. 204 AJ women diagnosed with invasive BC diagnosed <65 yrs 1986-1995 were studied. Pathology blocks were reviewed and immunohistochemical studies were undertaken. Follow-up was determined from chart review. DNA extracted from the blocks was analysed for the 3 common founder AJ mutations (185delAG, 5382insC and 6174delT). There were 32 BRCA mutation carriers (15.7%), 24 in BRCA1 and 8 in BRCA2. The median follow up was 76 months. At five years, there were 30 breast cancer deaths (14.7%) and 37 deaths from any cause. Tumor size (P=0.008), grade (<0.0001), ER (<0.0001) age (0.001), P53 status (0.018), HER2 status (0.056) and P27 status (0.009) were all associated in the expected ways with a worse prognosis. Women with BRCA mutations fared much worse: 5 year BC-specific mortality = 42.4% vs. 13.5% for non-carriers (P=0.0058). The difference in all-cause mortality was more striking: 38.9% vs. 15.7% (P=0.0006). BRCA1 and 2 did not differ in effect. In a univariate Cox model, the RR for dying of BC within 5 years of diagnosis in association with a BRCA mutation was 2.9 (1.3-6.3, P=0.008), but multivariately, BRCA status was not a significant factor (RR=1.2, P=0.66). However, removing grade (which is highly correlated with BRCA status) from the multivariate model resulted in a RR of 2.9, P=0.009. Importantly, studying node negative (NN=119) and node positive (NP=71) women separately, the multivariate RR was 2.5, P=0.15 for NN, BRCA+ women and 0.6, P=0.53 for NP, BRCA+ women; indicating that NN and NP women should be considered separately in survival analyses.

Program Nr: 323 from the 1999 ASHG Annual Meeting

Detection of heterozygote deletions within the hMLH1 gene using multiplex PCR of short fluorescent fragments.
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Detection of heterozygote deletions involving one or several exons represents a technical problem in autosomal dominant disorders, limiting the efficiency of mutations screening, and requires Southern blot analysis which is DNA and time consuming. We and others have identified in the hereditary nonpolyposis colorectal cancer (HNPCC) syndrome, large genomic Alu-mediated deletions within the hMLH1 gene. To facilitate the detection of such heterozygote deletions, we developed a simple semi-quantitative procedure based on the multiplex PCR of short fluorescent fragments. Short exonic fragments (92 to 296 pb) of the 19 hMLH1 exons, were PCR-amplified from genomic DNA using dye labelled primers. Exons 1 to 10 and 11 to 19 were PCR-amplified in two separate tubes. PCR products were loaded on a 4.25% denaturing polyacrylamide gel,electrophoresis was performed for 3 h on an Applied Biosystems model 377 automated sequencer, and data were analyzed using the Gene scanner Model 672 Fluorescent Fragment Analyser (Applied Biosystems, Perkin-Elmer). The concentration of genomic DNA and of the primers, the annealing temperature, the number of cycles, were optimized in order to obtain a very reproducible pattern between each sample. Reduction of the fluorescent intensity of the corresponding peaks allowed us to detect easily a deletion of exon 2 and a deletion of exon 13 to 16 in two French HNPCC families. This simple procedure can be applied to genes which inactivation might be mediated by exonic deletions.

Program Nr: 324 from the 1999 ASHG Annual Meeting

The rate of the founder Jewish mutations in BRCA1 and BRCA2 in prostate cancer patients in Israel. *E. Friedman[1], A. Vazina[1,2], A. Shtriker[1,2], Y. Yaacobi[1,2], T. Tischler[2].* 1) Oncogenetics Unit, Inst Gen, Chaim Sheba Medical Ctr, Tel Hashomer, Israel; 2) Departments of Urology and Oncology, Rabin, Wolfson and Sheba Medical centers, Israel.

Inherited predisposition occurs in 5-10% of all prostate cancer (CaP) patients, but the genes involved in conferring genetic susceptibility remain largely unknown. Several lines of evidence indicate that germline mutations in BRCA1 and BRCA2 might be associated with an increased risk for CaP. Three mutations in these two genes (185delAG and 5382InsC (BRCA1) and 6174delT (BRCA2)) occur in about 2.5% in the general Ashkenazi population, and the 185delAG BRCA1 mutation, in up to 1% of non-Ashkenazi Jews. In order to assess the contribution of these germline mutations to prostate cancer in Jewish Israeli patients, we tested 174 unselected prostate cancer patients (95 of Ashkenazi origin) for these mutations by PCR amplification and modified restriction enzyme digests. Patients age range was 45-81 years (median 66), and in 24 (14.4%) the disease was diagnosed before 55 years of age. Nineteen (11%) and 12 (6.9%) patients had first or second degree relative with CaP or breast cancer, respectively. Overall, 5 mutation carriers were detected: 2/152 (1.3%) 185delAG, 2/104 (2%) 5382InsC, and 1/158 (0.6%) 6174delT. In all carriers, the disease was diagnosed after the age of 55, and only one of them had a family history of breast and CaP. In addition, no allelic losses at the BRCA1 locus were demonstrated in 17 patients with a family history of CaP, using 7 microsatellite markers. We conclude that the rate of the predominant Jewish BRCA1 and BRCA2 mutations in CaP patients does not significantly differ from that of the general population, and that mutational inactivation of the BRCA1 is rare in familial CaP. Thus, germline BRCA1 and BRCA2 mutations probably contribute little to CaP occurrence, to inherited predisposition, and to early onset disease in Jewish individuals.

Program Nr: 325 from the 1999 ASHG Annual Meeting

Retinoblastoma treatment in premature infants diagnosed prenatally by ultrasound and molecular analysis. *B.L. Gallie[1,2], J.A. Gardiner[1], A. Toi[2], E. Héon[1,2], H. Chan[1], J. Sutherland[1], L. MacKeen[1], J. Anderson[1], L. Han[1], A. Budning[1], M. Sermer[2]*. 1) Ophthal/Ped, Div Immunol CA, Hosp Sick Children, Toronto, ON, Canada; 2) University Health Network, University of Toronto, Toronto, Canada.

The long-term visual outcome of two infants with familial retinoblastoma (RB) has been improved by prenatal diagnosis and treatment of vision-threatening tumors before expected birth-dates. One infant was diagnosed by prenatal ultrasound and one by molecular identification of germline RB gene mutation. Both were prematurely delivered at 36 weeks gestation for prompt treatment of their macular tumors. The infant with a large macular tumor observed to grow rapidly over one week, and a smaller macular tumor in the other eye, was treated with chemotherapy and laser. She will have a central scotoma in the eye with the large macular tumor, and normal central vision in the eye with the smaller macular tumor. The other infant had a tiny paramacular tumor at birth, and subsequently developed five tiny tumors, all of which are treated successfully with laser. She will have normal central vision in both eyes. If allowed to deliver at term, both infants would have had much larger macular tumors with significant loss of central vision despite more intensive therapy.

Program Nr: 326 from the 1999 ASHG Annual Meeting

Association of the C677T polymorphism in the MTHFR gene with breast and/or ovarian cancer risk in Jewish women. *R. Gershoni-Baruch[1,2], E. Dagan[1, 2], D. Israeli[3], E. Friedman[3].* 1) Dept Human Genetics, Rambam Medical Ctr, Haifa, Israel; 2) Bruce Rappoport Faculty of Medicine, Technion Institute of Technology, Haifa, Israel; 3) Susanne Levy Gertner Oncogenetics Unit, Chaim Sheba Medical Center, Tel-Aviv, Israel.

Methylenetetrahydrofolate reductase (MTHFR) catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5 methyltetrahydrofolate the primary circulatory form of folate and carbon donor for the re-methylation of homocysteine to methionine. A common missense mutation (C677T) in the MTHFR gene is associated with reduced enzyme activity, hyperhomocysteinemia and increased risk for atherosclerosis. Recently, a marginal association of the C677T polymorphism with endometrial and colorectal cancer was observed. To delineate the putative role of the C677T polymorphism in breast/ovarian tumorogenesis we determined the frequency of this polymorphism in 491 Jewish women with either sporadic (n = 355), hereditary (n = 136) breast and/or ovarian cancer who were all previously genotyped for the three predominant Jewish founder mutations in BRCA: 185delAG, 5382insC and 6174delT. Sixty nine asymptomatic BRCA mutation carriers were similarly analyzed. We found that C677T homozygotes were equally distributed among women with either sporadic (71/355; 20%) or hereditary breast/ovarian cancer (43/ 205; 21%); among women diagnosed with breast cancer prior to age 42 (22/122; 18%) and after that age (42/243; 17.3%); and among BRCA mutation carriers either asymptomatic (11/69; 15.9%) or manifesting cancer (32/136; 23.5%). Among women with bilateral breast cancer and those with both breast and ovarian carcinomas the rate of C677T homozygotes (24/72; 33.3%) was significantly higher (p = 0.0026). This observation, namely, that C677T homozygotes are at greater risk of acquiring a second primary tumor, if further corroborated has important clinical implications.

Program Nr: 327 from the 1999 ASHG Annual Meeting

A large genomic deletion of *hMLH1* in a family with Muir-Torre syndrome. *J.J.P. Gille[1], M.H.P. Strunk[1], R.J. van Schooten[1], L. Jaspars[2], M.H. Vermeer[3], G. Pals[1], F.H. Menko[1].* 1) Dept. of Clinical Genetics and Human Genetics; 2) Dept. of Pathology; 3) Dept. of Dermatology, University Hospital Vrije Universiteit, Amsterdam, The Netherlands.

Muir-Torre syndrome (MTS) is an autosomal dominant condition characterized by sebaceous gland tumors and visceral malignancies. In kindreds diagnosed with HNPCC (hereditary nonpolyposis colorectal cancer) sebaceous gland tumors and other MTS-associated skin tumors have been recognized. HNPCC is often due to germline mutations in one of five DNA-mismatch repair (MMR) genes (*hMSH2*, *hMLH1*, *hPMS1*, *hPMS2*, and *hMSH6*). Among MTS kindreds 14 *hMSH2* and 2 *hMLH* have been reported in the literature. Evidently, MTS and HNPCC are overlapping syndromes.

We studied a family (C149) in which the index patient (II-1) had two primary colorectal cancers at the age of 32 years. His father (I-1) had recurrent skin lesions diagnosed as sebaceous adenomas, sebaceous epitheliomas, keratoacanthomas with sebaceous differentiation and squamous cell carcinoma. At the age of 58 years this latter patient developed colonic cancer. No other close relatives were diagnosed with large bowel cancer or skin tumors. MSI studies of the colonic tumors of both patients revealed the MSI-H (high) phenotype. Germline mutation analysis of *hMLH1* and *hMSH2* by single strand conformation analysis and direct sequencing revealed that I-1 was apparently homozygote for two frequently occurring *hMLH1* polymorphisms located in exon 8 (696A>G) and intron 14 (IVS14-19A>G), respectively. Surprisingly, II-1 was not a carrier of any of these two polymorphisms, indicating that both patients were in fact hemizygote and carriers of a (partial) deletion of the *hMLH1* gene. Hemizygosity was confirmed by analysis of CA-repeat markers intragenic (D3S1611) and closely linked to *hMLH1* (D3S2623). No transmittance of alleles from I-1 to II-1 was observed. Our results indicate that both affected relatives are carriers of a genomic deletion of *hMLH1* that encompasses at least exons 8-14. The family presented here is the first MTS family with a large genomic deletion of *hMLH1*.

Program Nr: 328 from the 1999 ASHG Annual Meeting

Mutational analysis of the RET proto-oncogene in 200 French MEN 2 families: a genotype-phenotype correlation. *S. GIRAUD[1], P. PIGNY[2], P. NICCOLI-SIRE[3], P. NIZARD[1], A. MURAT[4], M. BILLAUD[5], G.M. LENOIR[1], GETC[3]*. 1) Lab. de Genetique,Hospital E. Herriot,LYON,FRANCE; 2) Hospital Huriez,LILLE; 3) Hospital La Timone,MARSEILLE; 4) Hotel-Dieu,NANTES; 5) CNRS,UMR 5641,LYON.

Germline mutations of the RET proto-oncogene are associated with three inherited related disorders: multiple endocrine neoplasia type 2A (MEN 2A), type 2B (MEN 2B) and familial medullary thyroid carcinoma (FMTC). We have screened exons 8, 10, 11, 13, 14, 15 and 16 of RET in the germline DNA of 200 MEN 2 families. RET mutations have been identified in 99% of MEN 2A (101/102), 100% of MEN 2B patients (27/27). Mutations of RET were found in 91% of FMTC families (66/72) but in all FMTC families with at least three cases of MTC.The majority of MEN2A mutations identified in our series were missense changes located in the region coding for the extracellular cysteine-rich domain of RET: 86% of the mutations affected codon 634 in exon 11 and 10% involved either codons 609, 611, 618 or 620 in exon 10. Also, two single nucleotide substitutions were found in exons 13 and 14 (Y791F and V804M) in two MEN 2A cases. A unique mutation in exon 16 (M918T) within the RET tyrosine kinase has been identified in all cases. With regard to FMTC, mutations in exons 10 et 11 were found in 54%; of the cases. However, as previously described, the distribution of mutations was dissimilar to MEN 2A since cysteine codons of exons 10 and 11 were affected in 39% and 15%, respectively. Furthermore, a new RET mutation that consists in a nine base pair duplication in exon 8 which creates an additional cysteine codon was characterized in one FMTC kindred. Finally, point mutations at codons that specify residues within the tyrosine kinase domain were found in 35% of the cases: 8% at codon 768 or 790 in exon 13; 20% at codon 804 in exon 14 and 7% at codon 891 in exon 15. Notably, carriers of RET mutations in exons 13 to 15 were characterized by a later age of onset and a variable penetrance of medullar thyroid cancer.Finally, based on the results of our functional analyses we will propose a possible biochemical explanation for the correlation between genotype and phenotype.

Program Nr: 329 from the 1999 ASHG Annual Meeting

Renal Neoplasms in a Familial Multisystem Syndrome with Fibrofolliculomas as a Cutaneous Marker. *G.M. Glenn[1], M.M. Walther[1], J.R. Toro[1], S. Hewitt[1], P. Duray[1], P.L. Choyke[2], G. Weirich[3], M. Turner[1], W.M. Linehan[1], B. Zbar[3].* 1) Genetic Epidemiology Branch, Urologic Oncology Branch, Dermatology Branch, and Laboratory of Pathology, National Cancer Institute, Bethesda, MD; 2) Diagnostic Radiology Department, National Institutes of Health, Bethesda, MD; 3) Laboratory of Immunobiology, Frederick Cancer Research and Development Center, Frederick, MD.

In our studies of familial kidney neoplasms, we recognized a subset of families with renal tumors who were also affected by lung cysts, pneumothorax, and multiple cutaneous papules. In some family members, skin examinations, biopsies and dermatopathologic diagnoses were consistent with Birt-Hogg-Dube syndrome (BHD), a dominantly inherited predisposition to developing fibrofolliculomas, trichodiscomas, and acrochordons, but previously not known to be associated with internal neoplasms. We found renal neoplasms and BHD segregated together in an autosomal dominant pattern. To identify internal tumors, we performed CT scans of abdomen and pelvis with contrast, high resolution chest CTs, renal sonograms, and now have added colonoscopies to improve ascertainment of cases in families for linkage analysis. With referrals from dermatologists nationwide and abroad, we are studying 23 families with 79 individuals affected with BHD, of which 20 have renal epithelial neoplasms, 19 have spontaneous pneumothorax histories, and 12 have had colon polyps and/or colon carcinoma, and a colon tubulovillus adenoma has been seen. Distribution of renal tumor (RT) number in individuals from BHD families is: 1 RT in each of 6 individuals; 2-3 RTs in 4 individuals; and greater than 2-3 RTs in 10 individuals. Renal histopathologies included: Renal oncocytoma in 10 patients; papillary renal carcinoma in 4 patients; clear cell renal carcinoma in 4 patients; and chromophobe renal carcinoma in 2 patients. It is important to recognize the risk for benign and malignant internal tumors, and pneumothorax in individuals when there is a dermatologic diagnosis of BHD. The number and size of families we are studying should allow demonstration of the phenotypic spectrum and identification of the genetic basis of this genodermatosis associated multisystem and neoplastic syndrome.

Program Nr: 330 from the 1999 ASHG Annual Meeting

Association of oral contraceptives with breast cancer risk in a population-based sample of 426 breast cancer families. *D.M. Grabrick, J.R. Cerhan, F.J. Couch, R.A. Vierkant, T.M. Therneau, C.M. Vachon, J.E. Olson, V.S. Pankratz, L.C. Hartmann, T.A. Sellers.* Mayo Clinic, Rochester, MN.

Oral contraceptives (OCs) are weakly associated with an increased risk of breast cancer (BC) in the general population, but some data suggest a higher risk among BRCA1 and BRCA2 mutation carriers. This is clinically important as women in breast-ovarian cancer families may consider OC use to reduce their ovarian cancer risk. We analyzed data from the Minnesota Breast Cancer Family Study, a historical cohort study of relatives of 426 BC cases identified between 1944 and 1952, and followed through 1996. Ninety-eight percent of eligible families were recruited, and 93% of members participated. OC use and cancer incidence in sisters, daughters, granddaughters, nieces, and marry-ins were determined through telephone interviews. Through 1996, a total of 239 incident BCs were identified in the cohort of 6,150 women at risk. The lifetime prevalence of ever having used OCs was 51% overall and was similar for blood relatives and marry-ins (p=0.99). We used proportional hazards regression, accounting for birth cohort and correlated family data, to model the association between a time-dependent definition of OC use and age at onset of BC. The association of OC use with BC was examined within strata defined by degree of relationship to the proband, with never users as the reference category within each stratum. Among sisters and daughters, women who used OCs for 1 to 4 years were at 4.2-fold greater risk (95% C.I.: 2.1-8.6); for duration of use greater than 4 years the risk estimate was 2.2 (95% C.I.: 0.8-6.4). The corresponding risk estimates for granddaughters and nieces were 1.3 and 1.2, and for marry-ins 1.1 and 1.3, all nonsignificant. When analyses were repeated among the subset of families with 3+ breast or ovarian cancers, risks associated with OC use were further elevated among first-degree relatives: 5.5 (2.4-12.5) and 3.3 (1.0-11.0) for 1 to 4 and greater than 4 years, respectively. These data suggest that use of oral contraceptives may significantly increase risk of breast cancer among women with a family history of breast cancer, especially those with a strong family history.

Program Nr: 331 from the 1999 ASHG Annual Meeting

Constitutional chromosomal instability and predisposition to childhood solid tumors; a new syndrome? *B. Hirsch[1], S. Berry[1], B. Bostrom[1,2], S. Sencer[2]*. 1) Univ Minnesota Medical School, Minneapolis, MN; 2) Children's Hospitals and Clinics, Minneapolis,MN.

The association between chromosomal instability (CI) and predisposition to malignancy is well documented in a number of genetic disorders. However, there are currently only a few well defined syndromes in which CI data are integrated into diagnostic testing or therapy planning.

We here report four children, from three unrelated families, who may represent a novel genetic syndrome. Clinical findings include IUGR, microcephaly, skin pigmentation anomalies, and/or anal abnormalities. Three children (CIA1, CIB1,CIC2)developed Wilms tumors within the first 2 yrs.of life,and one child (CIC1, the older sibling of CIC2),a high grade astrocytoma at 2.5 yrs. After surgical resection, chemotherapy was given to all but CIC2. Two children succumbed to therapy-associated AML within one yr., one died from therapy associated pancytopenia and sepsis. CIC2, who was not given chemotherapy because of concern for hypersensitivity, is alive 5 months post surgery. The three who succumbed all received topoisomerase II inhibitors.

G-banded metaphase analysis from blood lymphocytes and/or skin fibroblasts revealed markedly elevated rates of chromosomal breaks and rearrangements, 50 fold or greater relative to laboratory norms. No recurring abnormality or breakpoint was detected between children; however "clonal" rearrangements were found within individual studies. The pattern and rates of CI were not characteristic of a known disorder. SCE rates were normal, i.e. not indicative of Bloom syndrome.

The etiology of this syndrome is unknown. No mutation of the NF1 gene in patient CIAC2 was found. Although there was no prior significant cancer history in these families, analyses of mismatch repair genes are planned. Constitutional CI is clearly a hallmark of this disorder. Recently, a sibling to CIB1 was born with IUGR,microcephaly and displaced anus. Cytogenetic analysis revealed marked CI, as a result of which this patient is being carefully monitored for tumor development.

Program Nr: 332 from the 1999 ASHG Annual Meeting

The "few-hits-and-run" concept: a new model of carcinogenesis providing a meaningful etiologic and pathogenic interpretation of all the curious features of Lynch syndrome. *N. Janin.* Département de Génétique, Hôpital Necker-Enfants Malades, Paris, France.

The genotype-phenotype relationship of Lynch syndrome displays many curious features which cannot be explained by the prevailing concepts of carcinogenesis and genetic predisposition to cancer. In order to explain these anomalies, we propose a model of replication error (RER)-associated carcinogenesis based on the simple idea that the RER mutator phenotype has only a short term viability in normal cells because the enormous mutation load sustained by RER+ cells spontaneously leads to their elimination through apoptosis or senescence. The proposed model states that RER+ carcinogenesis is divided into two clearly distinct evolutive phases: 1 a preliminary phase starting with the counter-selective loss of mismatch repair function, in which most clones with the RER mutator phenotype are eliminated through apoptosis or an accelerated aging process; 2 an explosive phase that is initiated only if mutations blocking apoptosis and senescence, rapidly acquired during the short lifespan of the non-transformed RER+ clones, eventually rescue one RER+ cell that gives rise to the malignant clone. The classical concepts of initiation and promotion are irrelevant to this model as we consider RER+ carcinogenesis to progress irreversibly and very rapidly once initiated. The "run-initiation" is the unorthodox several-step initiation process of the model that entails all the rate-limiting steps of carcinogenesis. We shall show how the few-hits-and-run model simply explains the tissue specificity and penetrance of Lynch syndrome by taking into account the physiological variations of two key parameters which modulate the probability of completing the run-initiation process once a stem cell has acquired mismatch repair deficiency. These parameters are X and N, the number of self-maintenance mitoses tolerated by RER+ stem cells and the number of mitoses in the proliferating compartment of the tissue, respectively. X defines the life span of the RER+ clone and N modulates the number of viable and potentially transformable daughter cells generated before the extinction of the non-malignant RER+ clone.

Program Nr: 333 from the 1999 ASHG Annual Meeting

Association of glutathione-S transferases m **and** q **null polymorphisms and malignant melanoma.** *P.A. Kanetsky[1,2], R. Holmes[2], A. Walker[1], D. Najarian[1], A. Halpern[3], D. Guerry[2], T.R. Rebbeck[1,2]*. 1) Department of Biostatistics & Epidemiology and; 2) Cancer Center, University of Pennsylvania, Philadelphia, PA; 3) Department of Dermatology, Memorial Sloan-Kettering Cancer Center, New York, NY.

Polymorphisms in glutathione-S transferases m (*GSTM1*) and q (*GSTT1*) are candidate cancer susceptibility genes because of their role in carcinogen metabolism. GST's are active in skin and may impact upon the development of malignant melanoma by reducing cellular oxidative stress. To test for an association between *GSTM1* and *GSTT1* null polymorphisms (*GSTM1*0* and *GSTT1*0*, respectively) and melanoma, patients with incident melanoma (n=249) were accrued through the University of Pennsylvania Pigmented Lesion Clinic. Melanoma cases were compared to a group consisting of clinic patients with dysplastic nevi (DN) alone (n=98) and cancer-free controls (n=117) referred by clinic patients. Each participant completed a questionnaire that elicited information on demographics and cutaneous phenotype, and received a skin examination by a research nurse. DNA isolated from buccal swabs was amplified for *GSTM1* and *GSTT1* using polymerase chain reaction. After adjusting for age and presence of any DN, we found no association between melanoma and *GSTM1*0, GSTT1*0,* either *GSTM1*0* or *GSTT1*0,* or both *GSTM1*0* and *GSTT1*0*. However, among a subset of participants with blond or red hair, melanoma cases (n=56) were over 8-fold more likely [OR=8.9, 95% CI (1.1, 76)] to carry the null genotype for both *GSTM1* and *GSTT1* compared to the referent group (n=43). No associations between GST genotype and melanoma were noted after stratification on eye color, proclivity to burn in strong sun, ability to tan after repeated sun exposure, or freckling. These data suggest that among persons already at increased risk for melanoma, i.e. those with blond or red hair, GST m and q may act synergistically to further elevate cancer risk.

Program Nr: 334 from the 1999 ASHG Annual Meeting

Identification of a YAC carrying cell senescence gene for breast cancer. *G.P. Kaur[1], D.E. Reddy[1], C.L. Keck[2], N. Popescu[2], R.S. Athwal[1]*. 1) Fels Institute for Cancer Research and Molecular Biology, Temple Univ.Sch.Med., Philadelphia, PA 19140; 2) Laboratory of Experimental Carcinogenesis, NCI, Bethesda, MD 20892.

Loss of heterozygosity at chromosome 16q24 is frequently observed in breast cancer, suggesting the presence of tumor suppressor genes in this region. Previously, we have localized a cellular senescence gene at 16q24.3, within a genetic interval of 3-7 cM. A partial YAC contig of this genomic region was accessed. YACs, carrying chromosome 16 specific markers from senescence gene region, were modified to introduce neo selectable marker. Retrofitted YACs were first transferred into mouse A9 fibrosarcoma cell lines. Resulting A9-YAC hybrids were used as microcell donors to transfer YAC sequences into two immortal cell lines derived from human and rat mammary tumors. One YAC derivative, der792t2, restored senescence in both immortal cell lines, while an unrelated YAC from chromosome 6q, had no senescence activity. Human DNA present in A9-der792t2 hybrid, localized to chromosome 16q24.3 and 11q13 by fluorescent in situ hybridization. Since chromosome 11 does not induce senescence in any of recipient cell lines, we conclude that senescence activity is localized to 16q24.3 fragment in der792t2. Thus, using a functional approach, we have narrowed the senescence gene localization to a 360 kb of der792t2.

Program Nr: 335 from the 1999 ASHG Annual Meeting

BRCA1 germline mutations in women with uterine serous papillary carcinoma. *O. Lavie[1], G. Hornreich[1], A. Ben Arie[4], B. Kaufman[2], R. Catane[2], U. Beller[1], P. Renbaum[3], E. Levy-Lahad[3].* 1) Gynecologic Oncology; 2) Institute of Oncology; 3) Medical Genetics Unit, Shaare Zedek Medical Center, Jerusalem; 4) Dept. of Obstetrics and Gynecology, Kaplan Medical Center, Rehovot, Israel.

Uterine serous papillary carcinoma (USPC) is a distinct type of endometrial cancer which accounts for 3-4% of uterine malignancies. USPC histology resembles that of ovarian papillary serous adenocarcinoma, and is associated with a similarly adverse prognosis. In the Ashkenazi Jewish (AJ) population there is a high frequency (~30%) of founder BRCA1/2 mutations in women with ovarian cancer. In order to assess the role of BRCA1/2 mutations in USPC we studied 12 women consecutively diagnosed with USPC at two institutions. Nine were AJ, and 3 were Iraqi Jewish. Five patients (42%) had a personal history of breast cancer (brca) preceding the diagnosis of USPC, and 3 (25%, 2 AJ and one Iraqi Jewish) had a family history suggestive of familial breast-ovarian cancer. Genomic DNA samples from all patients were tested for the founder mutations (BRCA1 - 185delAG and 5382insC, BRCA2 - 6174delT). Two of nine (22%) AJ patients with USPC were found to be BRCA1 carriers (one 185delAG and one 5382insC). Both had a positive family history, and one was previously diagnosed with brca. DNA samples were obtained from the uterine tumors of both carriers and tested for loss of heterozygosity (LOH) at D17S855, an intragenic BRCA1 marker. LOH was observed in both tumors, suggesting USPC in these cases was causally related to the germline BRCA1 mutation. This preliminary study suggests that USPC can be a BRCA1-related malignancy. If substantiated in larger series, it may have implications for the prophylactic surgery offered to carriers, in that hysterectomy may need to be considered in conjunction with oophorectomy.

Program Nr: 336 from the 1999 ASHG Annual Meeting

PTCH Mutations in Squamous Cell Carcinoma. *P.K. Lee, X.L. Ping, H. Zhang, X.L. Wu, F.F. Chen, M.J. Zhang, D.N. Silvers, D. Ratner, M. Peacocke, H.C. Tsou.* Dermatology, Columbia University, New York, NY.

Squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) are classified as non-melanoma skin cancer (NMSC). Ultraviolet (UV) light exposure is the major risk factor for the development of SCC in Caucasians. Other risk factors include immunodeficiency, arsenic exposure and aging. Mutations in the tumor suppressor gene, p53, have been identified in SCC and BCC samples studied. Most of these mutations were UV specific C->T and CC->TT nucleotide changes, resulting in amino acid substitutions in the coding sequences of the p53 gene. PTCH, the human homologue of Drosophila patched gene, has also been shown to be mutated in sporadic BCCs. The role of the PTCH gene in SCC is unknown.

In this study, we screened a total of 20 SCC samples for mutations in the PTCH gene. We used PCR-SSCP as an initial screening method. Seven SSCP variants were detected in six SCC samples. Sequence analysis of these seven variants identified one nonsense mutation, three missense mutations and three silent mutations. In one SCC sample, we identified a tandem GG->AA transitional change at nucleotide 3152 in exon 18 of the PTCH gene, which resulted in a premature stop codon at codon 1051. In two other SCC samples, we identified UV specific C->T nucleotide changes in coding sequences of the PTCH gene. Sequence analysis of the p53 gene in these six SSC samples identified two CC->TT and four C->T UV specific nucleotide changes.

Our study provides the first evidence that PTCH is mutated in SCC and that mutations in both p53 and PTCH genes are found in the same SCC. The identification of the UV specific nucleotide changes in both tumor suppressor genes supports the notion that UV exposure plays an important role in the development of SCC.

Program Nr: 337 from the 1999 ASHG Annual Meeting

A Direct Interaction Between the EXT Tumor Suppressors and Glycosyltransferases. *M. Lovett[1], A. Simmons[2], C. Lopes[2], L. Hwang[2], Y. Yang[2], M. Musy[2].* 1) Washington University Medical Center, St.Louis, MO; 2) UT Southwestern Medical Center, Dallas, TX.

Hereditary multiple exostoses (HME) is an autosomal dominant disease in which bony outgrowths occur from the juxtaepiphyseal regions of the long bones. In a few percent of cases these exostoses undergo malignant transformation to chondrosarcomas. HME results from mutations in one of two homologous genes, EXT1 and EXT2 and loss of the wild type alleles of these genes appears to result in malignant transformation in a manner typical of tumor suppressors. EXT1 and 2 are members of a new gene family that is conserved from C. elegans to higher vertebrates. In humans this family comprises five genes of which only EXT1 and EXT2 are known to be involved in HME. The EXT genes are most conserved at their C-termini, but they do not contain any discernible motifs and their function(s) are unclear. Indirect evidence suggests that EXT proteins are involved in glycosaminoglycan (GAG) synthesis, and affect hedgehog signaling. There is also a report that these proteins co-purify with glycosyltransferase (GalNAc-T) activity and it has been postulated that they are themselves GalNAC-Ts. We performed two-hybrid screens with a fragment of EXT2 from the region that is most conserved in the gene family and identified two interacting proteins: the tumor necrosis factor type 1 receptor associated protein (TRAP1) and a novel glycosyltransferase, GalNAc-T5. TRAP1 is a distantly related member of the Hsp90 family of chaperonins that was previously reported to interact with the RB1 gene product and with the TNF1 receptor. TRAP1 interacted with both EXT1 and EXT2, but failed to interact with the related EXTL1 or EXTL3 proteins. By contrast, GalNAc-T5 interacted in vivo only with EXT2, but could also bind EXT1 protein in vivo. Significantly, both sets of interactions were abrogated by a disease-causing EXT mutation, indicating that they are important in HME. The EXT2:GalNAc-T5 interaction provides the first direct physical link between EXT proteins and glycosaminoglycan synthesis, suggests that EXTs are not themselves GalNAc-Ts and points to a potentially novel pathway of tumor suppression.

Program Nr: 338 from the 1999 ASHG Annual Meeting

Allelic loss at the four critical lung cancer tumour suppressor gene regions on human chromosome 3p including homozygous deletions at 3p12-13. *D.P. Macartney[1], A. Chauhan[2], S. Hosoe[3], E.R. Maher[1], F. Latif[1]*. 1) Division of Medical Genetics, Department of Paediatrics and Child Health, Birmingham University, Birmingham, UK, B15 2TT; 2) Cytogenetics and DNA Laboratory, Birmingham Womens Hospital, Birmingham, UK, B15 2TG; 3) Department of Internal Medicine, National Kinki-Chou Hospital, 1180, Nagasonecho, Sakai, Osaka, 591, Japan.

Cytogenetic and allelotyping analyses have revealed that human chromosome 3p is the most frequent site of allele loss in lung cancer and that this loss is observed in the earliest stages of the disease. Four distinct regions of loss at 3p12-13, 3p14.2, 3p21.3 and 3p25 have been identified which are potential sites for tumour suppressor genes involved in lung cancer. We have analysed these four regions using 12 microsatellite markers in 36 SCLC (small cell lung cancer) and 51 NSCLC (non-small cell lung cancer) normal/tumour pairs. We confirmed that 3p allele loss is more frequent in SCLC than NSCLC. Loss of heterozygosity was observed in all four regions for SCLC and NSCLC with no relationship seen between tumour type and a specific critical region. To date homozygous deletions have been reported in SCLC cell lines and NSCLC tumours with squamous histology at 3p21, in cell lines at 3p14.2 and in one SCLC cell line and one SCLC tumour at 3p12. Our results, based on the determination of homozygous deletion by the apparent retention of one microsatellite marker flanked by two markers showing clear loss (Cairns *et al.* 1995; Reed *et al.* 1996), strongly suggest the identification of two homozygous deletions, one of 1cM or less in a SCLC tumour at 3p12 and one of 30cM or less in a NSCLC tumour at 3p12-13. Quantitative PCR is being used to confirm the data and to fine map the deletions which may allow the 3p12 critical region to be narrowed further. In addition, when confirmed this will be the first report of a homozygous deletion in a NSCLC tumour at 3p12-13 further establishing the potential importance of this region in the pathogenesis of both SCLC and NSCLC.

Program Nr: 339 from the 1999 ASHG Annual Meeting

BRCA1/BRCA2 mutations in relation to tumor characteristics and prognosis. *E.A. Ostrander, K.E. Malone, P.L. Porter, D. Doody, M.G. Lin, N. Suter, J.R. Daling.* Fred Hutchinson Cancer Research Center, Seattle, WA.

The possibility that inherited susceptibility may influence tumor characteristics and/or prognosis has important implications for treatment choices and for understanding the natural history and progression of breast cancer. Using a population-based series of breast cancer cases diagnosed before age 45 in Washington State who are the focus of partially overlapping studies of both the genetic-epidemiology of the disease and prognostic factors, we assessed the relation of germline mutations in BRCA1 and BRCA2 to prognosis and tumor characteristics. 386 cases deemed to be at increased risk of carrying mutations, 203 diagnosed before age 35 unselected for family history, and 225 diagnosed before age 45 who had a first degree family history, were tested for mutations. Among the 387 cases, we found that BRCA1 positive cases (n=23) had a 50% reduction in the risk of dying (10 year survival) compared to BRCA1-/BRCA2- cases. BRCA2 positive cases (n=15) had an increased risk of dying (RR=2.5). As part of a study of prognostic factors for invasive breast cancer, we have obtained tumors from 217 of the 387 cases genotyped for BRCA1/2. Compared to BRCA1-/BRCA2- cases and compared to all cases in the prognosis study, the 13 BRCA1 positive tumors studied had adverse characteristics (92.3% ER-, 100% PR-, 73.3% high grade, etc.). BRCA2 positive tumors had similar tumor profiles to BRCA1-/BRCA2- cases and to the distribution in all invasive breast cancer cases. Finally, as reported in some other studies, we find that BRCA1, but not BRCA2 mutations are disproportionately related to a higher percentage of medullary breast cancers. These data suggest a need for larger, more extended analyses to better understand the relationship between inherited predisposition and natural history of disease.

Program Nr: 340 from the 1999 ASHG Annual Meeting

hMSH6 Alterations in Patients with MSI-L Colorectal Cancer. *Y.R Parc, K.C Halling, L. Wang, E.R Christensen, J.M Cunningham, L.J Burgart, T.L Price-Troska, S.N Thibodeau.* Departement of Laboratory Medecine and Pathology, Mayo Clinic, Rochester, MN.

Most hereditary nonpolyposis colorectal cancer (HNPCC) patients have germline mutations of one of the DNA mismatch repair genes, most frequently hMSH2 or hMLH1. It has been reported that some HNPCC families with low level MSI (MSI-L, MSI at 1-30% of the loci examined) have germline mutations of the hMSH6 gene. However, the role of hMSH6 in unselected MSI-L colon carcinoma is unknown. The aim of this study was to determine the frequency of hMSH6 mutations in patients with unselected CRC with MSI-L. Tumors from 46 patients with MSI-L colorectal cancer, derived from 404 unselected colon cancer, were screened for hMSH6 mutations using conformation sensitive gel electrophoresis (CSGE) and for an absence of hMSH6 protein expression by immunohistochemistry. Alterations found with CSGE were confirmed by DNA sequencing on normal and tumor tissue. One somatic (Asp389Asn) and 16 germline changes were found. Of the 16 germline changes, 9 were intronic (none involving the splice junction), and 7 were exonic (3 missense: Gly39Glu; Leu395Val; Ser503Cys and 4 silent alterations). One intronic alteration, an 18 bp deletion within the branch site of intron 7 (34 bp upstream from exon 8), was found in eight patients. However, these patients demonstrated a normal RNA expression by northern blot. None of the three patients with germline missense mutations had a family history consistent with or suggestive of HNPCC. Allele frequencies of these three alterations were 1%, 2% and 1%, respectively. The codon 39 alteration has already been reported as a polymorphism (Genomics 1996;31:395), the codon 395 alteration was found in 2 of 50 normal control patients, but the codon 503 alteration was not found in any of the 50 normal controls. The patient that met the Amsterdam criteria demonstrated the 18 bp deletion in intron 7 and a silent mutation in exon 3. Immunohistochemical staining for hMSH6 revealed no evidence of loss of hMSH6 expression in any of the 46 tumors. Our results suggest that hMSH6 mutations are minimally involved in the development of sporadic colorectal cancer with an MSI-L phenotype.

Program Nr: 341 from the 1999 ASHG Annual Meeting

Somatic Mutations in the SRD5A2 Gene Encoding Prostatic Steroid 5alpha-Reductase During Prostate Cancer Progression. *J.V. Reichardt, A. Akalu.* Biochem, Inst Genetic Medicine, Univ Southern CA Sch Medicine, Los Angeles, CA.

Prostate cancer will strike about 180,000 men in this country this year alone and almost 40,000 will die of the disease. Prostate cancer is an androgen-dependent disease which often progresses from androgen-dependent to androgen-independent growth. We have investigated the SRD5A2 gene encoding the prostatic (or type II) steroid 5alpha-reducatase which is centrally involved in androgen-activation in the prostate. We had previously shown that this gene undergoes frequent LOH (loss of heterozygosity) and genomic instability events during tumor progression. We report here our systematic analyses of the SRD5A2 gene in 30 matched tumor and constitutional DNA samples from men with prostate cancer. We identified at least two hot spots for somatic mutations: 1) the A49T (alanine at codon 49 to threonine) mutation which was found to occur de novo in 7 of 30 tumors and 2) the V63M substitution (valine-63 to methionine) which we identified as a de novo somatic mutation in 3 out of 30 tumor specimen. These two mutational hot spots, therefore, are found in one third of prostate tumors examined. Our data supports the notion that somatic mutations of the SRD5A2 gene may play an important role in prostate cancer progression. These data have important implications for the management of prostate cancer and its chemoprevention.

Program Nr: 342 from the 1999 ASHG Annual Meeting

Biochemical characterization of genetic variants identified during BRCA1 and BRCA2 clinical mutation screening for effects on RNA splicing. *T. Scholl, M. Pyne, D. Pruss, B. Ward.* Product Development, Myriad Genetic Laboratories, Salt Lake City, UT.

Certain variants detected in BRCA1/2 clinical testing could be predicted to interfere with RNA splicing based on location or sequence context. Their clinical significance was determined by using coding region polymorphisms to assess the chromosomal contribution to normal and aberrant RNA splicing products. Sixteen genetic variants occurring near intron-exon junctions within BRCA1/2 were evaluated. Nine represent mutations since the chromosome carrying the variant did not produce detectable levels of normal mRNA or produced only aberrantly spliced transcript(s): B1IVS4-1G->T, B1IVS8+2T->A, B1IVS16+6T->C, B1IVS18-13A->G, B1IVS19-2A->G, B1IVS22+2delT, B2R2336P, B2IVS7+2T->G, B2IVS17-1G->C; seven are polymorphisms since both chromosomes produced the legitimate message: B1C(TGC)197C(TGT), B1IVS8-57insT, B1IVS17ins1, B1IVS20+48ins12, B1IVS22+8T->C, B2IVS25-12T->G, B2IVS26-19G->A. SNP haplotype analysis was used to confirm conclusions based on biochemical data and also supported a reinterpretation of results for the polymorphism, B1IVS20+48ins12, which had previously been reported as a potential mutation. This combined approach to variant characterization produces very informative data since the contribution by either chromosome to every RNA species can be determined. Therefore, complications arising from the assessment of alternative versus aberrant splicing products are eliminated. The biochemical data is augmented with a haplotype analysis that resolves the relationship between genetic variants and aberrant transcripts produced by one chromosome. This method offers the potential to determine the clinical significance of variants that impact splicing using a sample from only a single patient and thereby obviate the requirement for an extensive familial or genetic study. The application of this approach has improved the clinical utility of the results presented to over 100 individual patients and permitted the unequivocal diagnosis of the presence of a deleterious mutation in thirty of these patients.

Program Nr: 343 from the 1999 ASHG Annual Meeting

Sex-specific expression of gastrin-releasing peptide receptor is related to smoking history and risk for lung cancer. *S.P. Shriver[1,2], H.A. Bourdeau[1], C.T. Gubish[1], D.L. Tirpak[1], A.L. Gaither Davis[1], J.M. Siegfried[1]*. 1) Department of Pharmacology, Lung Cancer Program, University of Pittsburgh Cancer Institute, University of Pittsburgh, Pittsburgh, PA 15261, USA; 2) Department of Biology, The Pennsylvania State University, 208 Mueller Lab, University Park, PA 16802.

Increasing evidence demonstrates that women are more susceptible to tobacco carcinogenesis than men. Activation of the gastrin-releasing peptide receptor (GRPR) in the human airway has been associated with long-term tobacco use, and with proliferative response of bronchial cells to gastrin-releasing peptide. The GRPR gene is located on the X chromosome and escapes X-inactivation. We hypothesized that expression of GRPR in the airway may be associated with susceptibility of women to the effects of tobacco carcinogens. GRPR mRNA expression was analyzed in lung tissues and cultured airway cells from 78 individuals, and in lung fibroblasts exposed to nicotine. Labeled nicotine and nicotine antagonists were used to assay airway cells for nicotinic acetylcholine receptors. A polymorphism located in exon 2 of the GRPR gene was used to detect allele-specific GRPR mRNA expression. GRPR mRNA is expressed in airway cells and tissues from female nonsmokers and short-term smokers at a significantly higher frequency than in males (£25 PY (pack-years); p=0.01); and female smokers exhibit expression of GRPR at a significantly lower mean pack-year exposure than males (56.3 PY for males vs. 37.4 PY for females, p=0.037). Elevated GRPR mRNA expression in lung fibroblasts was observed following exposure to nicotine, and lung fibroblasts and bronchial epithelial cells exhibited high-affinity, saturable nicotinic acetylcholine binding sites. Polymorphism analysis demonstrates that GRPR expression in women is the result of transcription from both copies of the gene. Our results suggest the GRPR gene is expressed more frequently in women in the absence of smoking, and/or that expression is activated earlier in response to tobacco exposure compared to men. This effect may be mediated by nicotine. The second expressed copy of the GRPR gene in females may be a factor in the increased susceptibility of women to tobacco-induced lung cancer.

Program Nr: 344 from the 1999 ASHG Annual Meeting

BRCA1 and _BRCA2_ germline mutations in a population-based series of early onset breast cancer patients in France. *O.M. Sinilnikova[1,2], V. Bonadona[3], S. Chopin[1], A. Brémond[3], H. Mignotte[3], P. Mathevet[2], A. Martin[4], C. Zinzindohoue[2], D. Raudrant[2], R-C. Rudigoz[2], C. Palayer[5], P. Romestaing[2], C. Montvernay[2], G. Lenoir[1,2], C. Lasset[3]*. 1) International Agency for Research on Cancer, Lyon; 2) Hospices Civils de Lyon, Lyon; 3) Centre Léon Bérard, Lyon; 4) Clinique Jeanne d'Arc, Lyon; 5) Clinique Eugène André, Lyon, France.

To study the contribution of germinal *BRCA1* and *BRCA2* mutations in breast cancer in the general population we have analyzed for mutations in these genes a population-based series of early-onset breast cancer patients from France of the Rhône administrative region. 457 women diagnosed with breast cancer before the age 45 in the Rhône region through the years 1995-1997 have been identified through a population-based cancer registry and 265 (60%) have already agreed to participate in the study and were interviewed, providing information on personal and familial cancer risk factors. The participation rate was slightly higher in the youngest age at diagnosis groups : 79%, 67% and 59% in the before 36, 36-40 and 41-45 age groups, respectively. 225 patients have already donated their blood. Here we report the results of the *BRCA1* and *BRCA2* mutation screen in 76 patients diagnosed with breast cancer before 40 years of age. The entire coding sequences and splice junctions of *BRCA1* and *BRCA2* have been analyzed by heteroduplex mutation detection technique. Eleven *BRCA1* and seven *BRCA2* mutations have been identified : ten mutations resulting in generation of a premature Stop codon, four missense variants, one in-frame deletion and three intronic variants expected to result in aberrant splicing. Our results indicate that a substantial proportion of young breast cancers (£40 years) drawn from the general population is attributable to mutations in the *BRCA1* and *BRCA2* genes (24% (18/76); truncating mutations only 13% (10/76)). 11% (5/46) of patients revealing no family history of breast or ovarian cancer were found to be *BRCA1* or *BRCA2* mutation carriers. Our data argue for utility of *BRCA1* and *BRCA2* mutation test in early-onset breast cancer patients unselected for family history of cancer.

Program Nr: 345 from the 1999 ASHG Annual Meeting

Polymorphisms in the methylenetetrahydrofolate reductase gene and susceptibility to leukemia. *C.F. Skibola[1], M.T. Smith[1], E. Kane[2], E. Roman[2], S. Rollinson[3], R. Cartwright[2], G. Morgan[2].* 1) School of Public Health, U.C. Berkeley, Berkeley, CA; 2) Leukaemia Research Fund Centre for Clinical Epidemiology, Leeds, UK; 3) Department of Haematology, University of Leeds, Leeds, UK.

Leukemias most likely arise through adverse gene-environment interactions with susceptibility being related to polymorphisms in multiple genes. We investigated the possible association between polymorphisms in the folate metabolizing enzyme, 5,10-methylenetetrahydrofolate reductase (MTHFR), and leukemogenesis. MTHFR catalyzes the reduction of 5,10-methylenetetrahydrofolate (methyleneTHF), a methyl donor for DNA synthesis, to 5-methyltetrahydrofolate (methylTHF), the primary methyl donor for methionine synthesis. A common 677 C®T polymorphism in the MTHFR gene results in thermolability of the enzyme that decreases the pool of methylTHF and increases the pool of methyleneTHF in homozygous mutants (677TT). Recently, another common 1298 A®C polymorphism has been identified in the MTHFR gene that results in diminished enzyme activity. We tested whether carriers of these variant alleles are protected from adult acute leukemia. We analyzed DNA from a case-control study in the United Kingdom of 308 adult acute leukemia patients and 491 age and sex matched controls. MTHFR variant alleles were determined by a PCR-RFLP assay. The MTHFR 677TT genotype was lower among 71 acute lymphocytic leukemia (ALL) cases compared to 114 controls, conferring a 4.3-fold decrease in risk of ALL (OR= 0.23; 95% CI= 0.06-0.81). We observed a 2.8-fold reduction in risk of ALL in individuals with the MTHFR 1298AC polymorphism (OR=0.33; 95% CI = 0.15-0.73), and a 14-fold decreased risk of ALL in those with the MTHFR 1298CC variant allele (OR=0.07; 95% CI= 0.00-1.77). In acute myeloid leukemia (AML), no significant difference in MTHFR 677 and 1298 genotype frequencies was observed between 237 cases and 377 controls. Individuals with the MTHFR 677TT, 1298AC and 1298CC genotypes have a decreased risk of adult ALL, but not AML, which suggests that folate metabolism plays a key role in the development of ALL.

Program Nr: 346 from the 1999 ASHG Annual Meeting

BRCA1 mutation screening using morphoclinical features of breast cancers. *H.H. Sobol[1], F. Eisinger[1], C. Nogues[2], M. Champeme[2], I. Bieche[2], D. Birnbaum[1], C. Pallud[2], J. Jacquemier[1], R. Lidereau[2]*. 1) Department of Genetic Oncology, Inserm E9939, Paoli Calmettes Inst, Marseille, France; 2) Genetic Oncology Unit, Rene Huguenin Centre, Saint-Cloud, France.

BRCA1-breast cancers display a specific morphoclinical pattern. The three most discriminant parameters are young age at onset, oestrogen receptor negativity (ER-) and poor differentiation (TD3) of the tumor. We tested the interest of these parameters in BRCA1 mutation search in a series of 65 consecutive invasive breast cancers with onset by the age of 35 years. A high BRCA1 mutation rate of 28.5 percent was found in tumors both ER- and TD3, versus only 3.9 percent in tumours with another profile (p=0.017; OR=9.8. When using early onset only the mutation rate was 9.2 percent. The family history of cases ER- and TD3 with a BRCA1 mutation was then investigated and none of them harbored an extensive family history of breast/ovarian cancer. Such a strategy would allow to identify gene carriers who would have been overlooked using current criteria such as family history of breast/ovarian cancer.

Program Nr: 347 from the 1999 ASHG Annual Meeting

C-abl, a downstream target of ATM signaling, localizes with ATM to synaptonemal complexes in meiotic spermatocytes. *W. Wang, M.S. Meyn.* Department of Genetics, The Hospital for Sick Children, Toronto, Ontario, Canada.

ATM mutations cause ataxia-telangiectasia (A-T), an autosomal recessive disease marked by neurodegeration, immunodeficiency, genetic instability, infertility, radiosensitivity and cancer. In the presence of double-strand DNA breaks (DSBs), ATM is thought to activate multiple cellular functions via kinase cascades. E.g., following irradiation, the proto-oncogene c-abl is phosphorylated in an ATM-dependent manner. C-abl in turn, can phosphorylate Rad51, a protein that plays a central role in genetic recombination. We previously demonstrated that ATM localizes to the synaptonemal complexes (SCs) of homologous chromosomes during meiosis I, a time when DSBs occur. *Atm -/-* and *C-abl-/-* mice are infertile, suggesting that both Atm and c-abl play critical roles in meiosis. Using immunohistochemical techniques, we have examined testes from normal and *Atm -/-* mice for the expression of c-abl, SCP3, ATM and rad51.

We confirm previous observations that c-abl protein is expressed in testes and now report that expression is highest in zygotene/pachytene spermatocytes. Beginning in mid-zygotene, discrete foci of c-abl are seen along the paired axes of SCs. These foci persist through early pachytene and colocalize with ATM and Rad51 at early recombination nodules. Pairing of meiotic chromosomes is grossly abnormal in *Atm -/-* mice, with meiosis I ending in fragmentation of SCs and apoptosis. Despite these abnormalities, formation of c-abl foci appears to be normal in *Atm -/-* spermatocytes. Unlike Rad51, no off-axis c-abl foci are observed in the degenerating pachytene *Atm -/-* nuclei.

Our studies indicate that c-abl forms foci along the SCs of paired chromosomes and suggest that c-abl is a component of early recombination nodules. In addition, functional Atm does not appear to be required for c-abl focus formation. These results, together with the demonstration of abnormal Rad51 foci in *Atm -/-* spermatocytes, suggests that the ATM/c-abl/Rad51 signaling pathway plays a critical role in regulating genetic recombination in both mitotic and meiotic cells.

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Program Nr: 348 from the 1999 ASHG Annual Meeting

Subtelomeric rearrangements detected by FISH in patients with idiopatic mental retardation. *B. Anderlid[1], G. Annerén[2], E. Blennow[1], M. Nordenskjold[1]*. 1) Dept of Clinical Genetics, Karolinska Institute, Stockholm, Stockholm, Sweden; 2) Dept of Clinical Genetics, Akademiska Hospital, Uppsala.

Introduction: Mental retardation (MR) affects 1-3% of the population. One important cause is chromosomal rearrangements altering the gene dosage. Although thoroughly investigated, many cases do not get an etiological diagnosis. The subtelomeric regions are gene rich and have a high degree of involvement in chromosomal rearrangements. Recent investigations showed submicroscopic subtelomeric rearrangements in 5-10% of cases with idiopatic MR. Material: 44 patients (23 male and 21 female), aged 1-30 years (median 7.9 years) with idiopatic MR were analysed. Severe MR was found in 27, 31 had dysmorphic features or malformations and 14 had heredity for MR. Standard chromosome analysis was normal in all. Methods: The subtelomeric regions were screened with fluorescent in situ hybridization (FISH). The Chromoprobe Multiprobe T System was used. It contains probes from all subtelomeric regions on a device making it possible to analyse all chromosomes on one slide. Probes from p-arms are labelled with digoxigenin and q-arms with biotin. The signals are amplified using antibodies (antidigoxigenin-FITC and antibiotin-Cy3)in several steps. Results: Subtelomeric rearrangements were found in 6 patients: Del 9q - a woman born 1977 with MR and autism. Del 21q,dupl 9q - a boy born 1984 with severe MR, short stature, VOC, CNS malformation and heredity for MR. Del 2q - a girl born 1988 with mild MR, dysmorphic features and a sister with MR. Del 22q - a woman born 1966 with MR, autism, epilepsy and dysmorphic features. Del 6q - a boy born 1995 with severe MR, microcephaly, hyperactivity, skeletal malformations and a brother with MR. Del 4p - a girl born 1996 with severe MR, reduction of white matter, epilepsy and dysmorphic features. A high number of cross hybridizations were detected. The most common were 11p-17p, 12p-6p and 12p-20q. Conclusion: Six out of 44 patients with idiopatic MR were found to have subtelomeric rearrangements not detected by routine chromosome analysis. This supports the hypothesis that subtelomeric rearrangements are an important etiological factor in idiopatic MR.

Program Nr: 349 from the 1999 ASHG Annual Meeting

MITF mutations in Waardenburg syndrome. *J. Augé[1], T. Attié-Bitach[1], B. Gilbert[2], M. Le Merrer[1], S. Lyonnet[1], A. Munnich[1], M. Vekemans[1]*. 1) Dept de Génétique & INSERM U-393, Hopital Necker, Paris, FRANCE; 2) Hopital Dupuytren, Limoges, FRANCE.

Waardenburg syndrome (WS) is a genetically heterogeneous disease associating congenital sensorineural deafness with skin irides and hair pigmentary disorders. WS has been classified in 3 types according to the presence (WS1) or absence (WS2) of dystopia canthorum. WS3 is more severe associating muscular limb defects to a WS1 phenotype. Mutations of the PAX3 gene are responsible for WS1 and WS3, while genetic heterogeneity is observed in WS2, where less than 20% MITF mutations are found. So far, ten different MITF mutations have been reported in 13 patients, 11 in WS2 cases and 1 in a family with a Tietz albinism-deafness syndrome (2 mutations identified twice). Here we report on the mutational spectrum in 31 WS families (20 WS1, 8 WS2 and 3 unclassified) analysed for PAX3 and/or MITF genes by single strand conformation polymorphism. We identified 8 PAX3 (4 novel mutations) and 4 MITF mutations. One MITF mutation has been identified in a WS2 patient with deafness, blue eyes, and grey hair. Her daughter is also affected with deafness and heterochromia of the irides. They carried a nonsense mutation (R214X) in exon 7 that has already been reported in two other patients with WS2. The 3 other mutations are novel MITF mutations. A missense mutation in exon 6 was identified in a patient with deafness, blue eyes, and a patchy hypopigmentated skin. His father has early greying and blue eyes but no DNA was available for studies. The causal nature of this missense mutation in uncertain, although lying in the basic domain and absent in 50 controls chromosome. An intron 7 MITF splice mutation was found in a large pedigree segregating for neurosensorial deafness, premature hair greying and irides heterochromia. Finally, an exon 8 2bp deletion was identified in a boy with deafness and heterochromia of the irides. The mutation was inherited from his mother who presented irides heterochromia, but was no deaf. In conclusion, this study reports 3 novel MITF mutations out of 13 mutations described so far and confirms the strong association of MITF mutations and WS2.

Program Nr: 350 from the 1999 ASHG Annual Meeting

Congenital Heart Disease in Monosomy 21: Definition of two distinct loci. *G.M. Barlow[1], X-N. Chen[1], G. Calabrese[2], G. Palka[2], P. Caldwell[3], C. Booth[4], L. Linck[5], J.R. Korenberg[1].* 1) Medical Genetics, Cedars-Sinai Medical Ctr, Los Angeles, CA; 2) University of Chieti, Italy; 3) Kaiser Permanente Punawai Clinic, Waipahu, Hawaii; 4) Lutheran General Medical Group, Chicago, IL; 5) Oregon Health Sciences University, Portland, OR.

Monosomy for chromsome 21 is usually associated with death in utero. However, individuals with partial deletions of chromosome 21 and congenital heart disease (CHD) provide the opportunity to identify genes contributing to cardiovascular development. To do this, we have defined the molecular structure and cardiac features of 16 subjects with partial deletions of chromosome 21. Thirty-eight markers spanning chromosome 21 were analysed using fluorescence in situ hybridisation with bacterial artifical chromosomes (BACs) or quantitative Southern blot dosage analysis. Of the sixteen individuals with partial monosomy 21, six (38%) have CHD. These are: case 1, who is deleted from APP-ITSN (intersectin) and has a VSD; case 2, who is deleted from SOD1-D21S17 and has a secundum ASD; case 3, who is deleted from KCNE-ter and has an ASD; case 4, who is deleted from ITSN-ter and has an ASD; case 5, who is deleted from CBR-ter and has complex CHD including a severe coarctation of the aorta, pulmonic stenosis, patent foramen ovale and hypoplastic left ventricle; and case 6, who is deleted from D21S121-DSCAM and also has complex CHD including a membranous VSD, an ASD secundum, anteriorly placed aorta, and patent ductus arteriosus. Combining the molecular results reveals that two deletions do not overlap (cases 1 and 5). Position effects notwithstanding, this suggests that deletions of two distinct regions of chromosome 21 can lead to CHD. Further, the region between CBR and the telomere that is associated with complex CHD overlaps the candidate region for congenital heart disease in trisomy 21 (Down Syndrome). We therefore propose that these regions contain dosage-sensitive gene(s) that are important for normal development of the heart and great vessels, and whose perturbation in aneuploidy 21 results in CHD.

Program Nr: 351 from the 1999 ASHG Annual Meeting

BOR syndrome. Report of seven family groups. *C. Bellini[1], D. Massocco[1], F. Scopesi[1], G. Piaggio[2], E. Verrina[2], F. Perfumo[2], F. Campone[1], W. Bonacci[1], G. Serra[1].* 1) Servizio di Patologia Neonatale, Universita di Genova, Istituto Gaslini, Genova, Italia; 2) Divisione di Nefrologia, Istituto Gaslini, Genova, Italia.

We review seven new families with branchio-oto-renal (BOR) syndrome (MIM 113650). BOR syndrome is an autosomal disorder with an incidence, probably understimated, of 1/40.000 cases. It consists of conductive, sensorineural, or mixed hearing loss; preauricular pits; structural defects of the outer, middle or inner ear; renal anomalies; lateral cervical fistulas, cysts or sinuses; nasolacrimal duct stenosis or fistulae; characteristic facial appearance. Facial paralysis has also been occasionally described. The minimal diagnostic criteria of BOR syndrome are unknown. In our study, all first diagnosed patients in each familial group were recognized on the basis of severe renal impairment, spanning from complete bilateral agenesis to bilateral or unilateral renal dysplasia. When evidentiated, kidneys were usually reduced in size. Severe renal insufficiency was demonstrated in all first diagnosed patients. On the basis of diagnosis of BOR syndrome, many family members of each family were evaluated. Parents of two patients were healthy without any marker of BOR syndrome. Pedigrees of the five other families showed presence of preauricular pits, branchial fistulae and hearing impairment at various degree. Severe renal impairment leading to death was recognized in one member among these families. Our study demostrated that BOR syndrome is an underdiagnosed disorder, usually recognized in the presence of severe renal failure, but often not diagnosed in the presence of mild clinical signs, such as mild branchial anomalies. We enphatize a meticolous search for renal anomalies and/or hearning loss in all subjects showing minimal signs of branchial defects; on the other hand, one BOR syndrome is diagnosed in a patient, it is necessary to examine all family members for the presence of the syndrome; and, this is true even if there are minimal stigmate of the disease in the family members.

Program Nr: 352 from the 1999 ASHG Annual Meeting

Clinical variability and genetic heterogeneity in multiple epiphyseal dysplasia. *M.D. Briggs[1], W. Newman[1], I.D. Young[2], G.R. Mortier[3].* 1) Wellcome Trust Centre for Cell Matrix Research, School of Biological Sciences, Univ. of Manchester; 2) Clinical Genetics Service, City Hospital, Nottingham, England; 3) Dept. Medical Genetics, Univ. Hospital of Gent, Gent, Belgium.

Multiple epiphyseal dysplasia (MED) is characterised by early-onset degenerative joint disease and in severe cases short stature. The mild 'Ribbing' and severe 'Fairbank' forms have been used to define the phenotypic spectrum within this autosomal dominant disease. MED can result from mutations in the genes encoding cartilage oligomeric matrix protein (COMP) and type IX collagen (COL9A2 and COL9A3). We are performing genetic linkage and mutational analysis in families with various MED phenotypes and comparing molecular findings to clinical and radiographic presentation. In a family with severe MED we identified a COMP mutation (D473N) that has also been shown to cause pseudoachondroplasia. In 2 families with mild MED, different mutations have been identified in the exon 3 splice-donor site of COL9A2, one of which results in the degradation of mRNA from the mutant allele. In another family in which COL9A2 is implicated, affected individuals are of normal stature, but osteochondritis dissecans has contributed significantly to early onset degenerative joint disease. Electron microscopy of cartilage from an affected family member shows normal extracellular matrix and chondrocyte morphology. Finally, we have ascertained a four generation family with MED in which preliminary genetic analysis has excluded linkage to the COMP or type IX collagen genes. Affected individuals have a normal birth length and an adult height around the third percentile (150-165 cm). Most have complained during childhood of knee and hip pain after exercise and some underwent hip replacements or knee surgery because of early-onset osteoarthritis. Current studies are focused on identifying the disease gene in this family and the genetic defect of MED in 5 other families. Our data suggest that mutations in at least five genes are likely to result in phenotypes within the MED disease spectrum, which helps to explain the clinical variability within this osteochondrodysplasia. (This work is funded by the Arthritis Research Campaign).

Program Nr: 353 from the 1999 ASHG Annual Meeting

Atypical microdeletion in 15q11-q13 re-establishing the regions for Angelman and Prader-Willi syndromes. *J.J. Burger, V. Wiebe, D. Horn, H. Tonnies, K. Sperling, A. Reis.* Human Genetics; Charite, Humboldt University, Berlin, Germany.

The genetic basis of Angelman syndrome (AS) is complex. AS appears to be caused by the loss of maternal UBE3A gene activity. Most AS patients have a 4 Mb de-novo deletion on their maternal chromosome 15q11-q13. If this deletion is of paternal origin, affected individuals develop Prader-Willi syndrome (PWS). We report an uncommon deletion of the UBE3A gene. It spans approximately 700 kb, encompasses the entire UBE3A gene, is flanked by microsatellites PAR/SN and D15S986, and is of maternal origin. The methylation pattern at the SNRPN locus is normal in Southern-Blot analyses. We confirmed the deletion with FISH using several BAC probes from the deleted region. We identified one clone spanning the distal breakpoint. We found the deletion also in the maternal grandfather and his sister, who has two children with disturbances compatible with AS. Our results suggest that the deletion occurred in the great-grandfather and that it causes AS when it is transmitted through a female germline. These findings support the hypothesis that the functional loss of the maternal UBE3A gene is sufficient to cause AS. Moreover, our results indicate that the deleted region does not contain any genes or other factors involved in PWS.

Program Nr: 354 from the 1999 ASHG Annual Meeting

A immunological method to detect mutations in the XNP gene, involved in several X-linked mental retardation syndromes. *C. CARDOSO[1], L. VILLARD[1], C. MIGNON[1], A.M. LOSSI[1], Y. LUTZ[2], J.L. MANDEL[2], M.G. MATTEI[1], C.E. SCHWARTZ[3], L. COLLEAUX[1], M. FONTES[1]*. 1) INSERM U 491, Faculté de Medecine de la Timone, 27 Bd J.Moulin, 13385 MARSEILLE cedex 05, FRANCE; 2) INSERM U 184, IGCBM, Parc d'innovation , BP 163 67404 Illkich cedex, FRANCE; 3) J.C. Self Research Institute, Greenwood Genetic Center, Greenwood, SC 29646, USA.

Mutations in the XNP gene, located in Xq13.3, cause several X-linked mental retardation syndromes : the a-thalassemia with mental retardation syndrome (ATR-X), Juberg-Marsidi syndrome, Carpenter Waziri syndrome, Holmes-Gang syndrome, Smith-Fineman-Myers syndrome, mental retardation with spastic paraplegia and mental retardation without a-thalassemia. Sixty-percent of mutations in XNP have been found within the zinc finger domain of the protein (encoded by exons 7,8 and beginning of exon 9) while 40% of the mutations were found in the helicase domain extending over 3 Kb at the C-terminus of the protein. All male patients reported so far have had severe mental retardation and dysmorphic features. In addition, patients may be affected with genital anomalies, Hb H inclusions, spastic paraplegia, club foot and optic anomalies. Thus, we anticipate that the presence of mental retardation and facial dysmorphy in a male will be the major criteria to consider a mutation in XNP as the causative factor. To study the function of the XNP gene product in normal and pathological conditions, we produced monoclonal antibodies against the XNP protein and performed immunocytochemical and western blot analyses on cells from various patients. Our results indicated that XNP expression was altered or absent in some cases. Based on these findings, we propose to use monoclonal antibodies directed against XNP to diagnose patients with mental retardation and mutations in this gene.

Program Nr: 355 from the 1999 ASHG Annual Meeting

Clinical features of the major form of childhood deafness, DFNB1, caused by a connexin26 gene defect. *F. Denoyelle[1,2], S. Marlin[1,2], D. Weil[2], E.N. Garabédian[1], C. Petit[2].* 1) Service d'ORL pédiatrique, Hôpital Armand-Trousseau, Paris, France; 2) Unité de Génétique des Déficits Sensoriels, Institut Pasteur, Paris, France.

DFNB1, an autosomal recessive form of deafness due to connexin26 gene (*CX26*) mutations, has been recently shown to be one of the most frequent hereditary defects in humans. To date, no clinical characterisation of the DFNB1 inner ear defects has been reported, which prevents the providing of prognostic information and proper genetic counseling. We searched for mutations in the non coding exon and in the coding region of *CX26* in 112 children (92 families) affected by sensorineural deafness with various degrees of hearing loss. The children either belonged to a family affected by autosomal recessive deafness (DFNB family) or represented sporadic cases. Audiometric, radiological and vestibular features were investigated and compared in deaf children with and without *CX26* mutations. Among the prelingually affected families, *CX26* mutations were present in 56.7% of DFNB families and 33.3% of the sporadic cases, versus none of the families with post lingual DFNB forms (p=0.01). The inner ear defects of 42 children carrying bi-allelic *CX26* mutations were compared with that of 50 prelingually deaf children without *CX26* mutations. DFNB1 deafness was mild to profound without genotype/phenotype correlation, associated to sloping or flat audiometric curves, radiologically normal inner ear and normal vestibular tests. The hearing loss was very rarely progressive. Frequent variations in the deafness severity between sibs were observed. The here described audiometric, vestibular and radiological characteristic features of DFNB1 would or should be the reference used to guide the order of *CX26* molecular diagnostic tests for deaf children with a compatible phenotype. A prognostic information can now be given to families: the hearing loss of the DFNB1 form of deafness is rarely progressive during childhood. A major element for the genetic counselling emerges: the severity of the hearing loss due to DFNB1 is extremely variable and cannot be predicted, even within families.

Program Nr: 356 from the 1999 ASHG Annual Meeting

A novel form of autosomal dominant movement disorder: familial dyskinesia and facial myokymia. *M. Fernandez[1], W. Raskind[1], J. Wolff[1,3], M. Matsushita[1], H. Lipe[3], T. Bird[1,2,3].* 1) Medicine and; 2) Neurology, Univ of Washington; 3) Veterans Affairs Puget Sound Health Care System, Seattle, WA.

Familial dyskinesia and facial myokymia (FDFM) is a novel autosomal dominant disorder characterized by adventitious movements that sometimes appear choreiform and that are associated with perioral and periorbital myokymia. The disorder has an early childhood or adolescent onset. The involuntary movements are paroxysmal at early ages, worsen, and may become constant in the twenties. Thereafter, there is no further deterioration and there may even be improvement in old age. The adventitious movements are worsened by anxiety, but not by voluntary movement, startle, caffeine or alcohol. The disease is socially disabling, but there is no intellectual impairment or decrease in lifespan. We report a four-generation family with 13 affected members, including 7 males and 6 females. We obtained samples from 9 affected and 3 unaffected members from three generations of this family. A simulation study suggested that the material available could provide a maximum lod score of 2.7. For this reason, a candidate gene approach was taken. We have focused on regions known to contain genes associated with chorea or myokymia. Using primers for polymorphic loci closely flanking or within genes of interest we excluded linkage to five regions: 1. The Huntington disease gene on chromosome 4p; 2. The paroxysmal dystonic choreoathetosis gene at 2q34; 3. The DRPLA gene at 12p13; 4. The choreoathetosis/spasticity disease locus on 1p that lies in a region containing a cluster of potassium (K^+) channel genes; 5. The episodic ataxia type 1 (EA1) locus on 12p that contains the KCNA1 gene and two other voltage-gated K^+ channel genes, KCNA5 and KCNA6. This latter area is of special interest not only because EA1 is caused by a K^+ channel gene mutation, but because the disease is also associated with myokymia. We are currently evaluating additional regions associated with chorea: 9q21 (chorea-acanthocytosis) and 20p (huntington-like disorder). Our results provide further evidence of genetic heterogeneity in autosomal dominant movement disorders and suggest a novel gene underlies this new condition.

Program Nr: 357 from the 1999 ASHG Annual Meeting

Age-related features of genetic mutations producing cognitive impairment: the Fragile X mutation, NF1, and Williams Syndrome. *G.S. Fisch[1], P.N. Howard-Peebles[2], N. Carpenter[3], J. Tarleton[4], R. Simensen[5], J.J.A. Holden[5]*. 1) Epidemiology & Public Health, Yale Univ School of Medicine, New Haven, Connecticut; 2) GIVF, Fairfax, and the Medical College of Viriginia, Richmond, Virginia; 3) Chapman Institute of Medical Genetics, Tulsa, Oklahoma; 4) Fullerton Genetics Center, Asheville, North Carolina; 5) Greenwood Genetics Center, Greenwood, South Carolina; Ongwanada Resource Centre, and Queens University, Kingston, Ontario.

Neurogenetic disorders of known etiology are valuable in that they provide a unique opportunity to study genotype-phenotype as well as brain-behavior relationships. Recently, our multicenter consortium investigated children and adolescents with the fragile X [fra(X)] mutation, noting longitudinal declines in composite IQ scores and adaptive behavior scores (DQ) in nearly all males with the full mutation and, to lesser extent, most fully mutated females. We were interested as to whether other genetic mutations of known etiology produced comparable age-related outcomes; or, whether the course of cognitive ability and/or adaptive behavior followed different temporal patterns. We obtained IQ and DQ scores, as well as maladaptive behavior scores, from children and adolescents diagnosed with the fra(X) mutation, Williams syndrome (WS), or Neurofibromatosis type 1 (NF1). Participants with NF1 (N=14) or WS (N=16) were age-matched with males diagnosed with the fra(X) mutation. IQ for all subjects was assessed with the Stanford-Binet 4th Edition; adaptive behavior (DQ) was evaluated using the Vineland Adaptive Behavior Scales. Our findings show strong and significant negative correlations between chronological age (CA) and either IQ or DQ among individuals with the fra(X) mutation and WS. Correlation between age and IQ in fra(X) was $r=-.74$ ($p<.005$); in WS, $r=-.73$; ($p<.005$). Correlation between age and DQ in fra(X) was $r=-.53$ ($p<.05$); in WS $r=-.86$ ($p<.001$). Unlike these individuals, children and adolescents with NF1 show modest positive correlations between age and IQ. All three etiological groups display significant age-related decreases in adaptive behavior scores. Additional analyses will be presented and discussed.

Program Nr: 358 from the 1999 ASHG Annual Meeting

A nonsense mutation of the *ATRX* gene causing mild, moderate and profound mental retardation in members of a single family. *R.J. Gibbons[1], R. Guerrini[2], J.L. Shanahan[1], R. Carrozzo[3], P. Bonanni[2], D.R. Higgs[4]*. 1) Nuffield Department of Clinical Biochemistry and Cellular Science, University of Oxford, John Radcliffe Hospital, Headington, Oxford, OX3 9DU, UK; 2) Institute of Child Neurology and Psychiatry, University of Pisa, and Institute for Clinical Research Stella Maris Foundation, Pisa, Italy; 3) Servizio di Genetica Medica e Laboratorio di Citogenetica, Ospedale San Raffaele, Milano, Italy; 4) MRC Molecular Haematology Unit, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, UK.

Mutations in the X-encoded gene *ATRX* are known to give rise to severe syndromal mental retardation. Here we describe a pedigree including 4 affected members aged 17 to 45 years. Two patients with the typical facial features of ATR-X syndrome have moderate (IQ41) and profound mental retardation (IQ unmeasurable on the Weschler scale) respectively. The other two presented with mild mental retardation (IQ56 and IQ58) and epilepsy but without the characteristic facial dysmorphism. While the two more severely affected boys had the characteristic facial features of ATR-X syndrome, there was no correlation between the degree of mental retardation and the severity of any other clinical or hematological features.

Mutation analysis identified a previously unreported 324C>T nonsense mutation in exon 2 of the *ATRX* gene in all four patients. No alternative splicing that might lead to the skipping of exon 2 could be detected in any of the affected individuals or normal controls. The explanation for the marked phenotypic variability between the affected individuals is as yet unexplained.

In the past, mutations in the *ATRX* gene have only been found in individuals with severe mental retardation and a variety of major developmental abnormalities. The striking phenotypical variability observed in this pedigree has important implications for counselling affected families and suggests that mutations in this gene should be considered as a cause of mild MR in male patients lacking specific diagnostic features.

Program Nr: 359 from the 1999 ASHG Annual Meeting

Madelung deformity in childhood is the result of SHOX haploinsufficiency. *I.A. Glass[1], S. Flanagan[1], M. Berry[2], D. Vickers[3], J.A. Batch[1], G. Rappold[4], V. Hyland[5]*. 1) Queensland Genetics Service and Department of Paediatrics, University of Queensland, Herston Hospitals Campus,Brisbane, Australia; 2) Mater Hospital Laboratory Services, Brisbane; 3) Department of Orthopaedics,Royal Childrens Hospital, Brisbane; 4) Institute of Human Genetics,University of Heidelberg, Germany; 5) Molecular Genetics, Department of Pathology, Royal Brisbane Hospital.

The madelung deformity is an abnormality of the forearm characterised by shortening and bowing of the radius with subluxation of the distal ulna. The exact proportion of madelung attributable to Leri-Weill syndrome(LWS),a sex-linked mesomelic skeletal dysplasia with disproportionate short stature, has historically been a contentious issue. Haploinsufficiency of the homeobox gene SHOX which maps to PAR1, appears to be the molecular basis of LWS. We have undertaken SHOX molecular analysis of 11 probands (10F,1M)who presented for orthopaedic surgery for correction of their madelung deformity. FISH analysis was carried out on metaphase spreads of peripheral lymphocytes. Cosmid LLNOYCO31M134F5 containing SHOX exons 1(part) to 5b was labelled with biotin, hybridised to the metaphases and detected by incubation with mouse monoclonal anti-biotin antibody,followed by goat anti-mouse-Oregon Green 488 antibody.We detected absence of SHOX signal in 6/11 probands,indicating large scale SHOX deletions were present. Further investigation of the 5 portion of SHOX exon 1 was performed using a 5intragenic SHOX dinucleotide repeat,but no further deletions were detected by this approach. Sequencing of theSHOX exons in the remainder of this cohort identified a patient harboring a heterozygous C to T transition(R195X). In one family, where the proband manifested a SHOX deletion, the demonstration of normal SHOX signals in a sibling, with equivocal radiological signs of early madelung deformity enabled this individuals status as a gene carrier to be clarified. We conclude that a substantial proportion of madelung deformity is due to LWS/SHOX haploinsufficiency and that molecular analysis of such cases may be useful for genetic counselling purposes.

Program Nr: 360 from the 1999 ASHG Annual Meeting

Expression of cholesterol biosynthetic enzymes is upregulated in Smith Magenis syndrome fibroblasts. *O. Goker-Alpan[1], J. Blancato[3], A.C.M. Smith[2,3], W.A. Gahl[1]*. 1) HDB, NICHD/NIH, Bethesda, MD; 2) NHGRI/NIH, Bethesda, MD; 3) Georgetown Univ., Washington DC.

Smith-Magenis syndrome (SMS), due to interstitial deletion of 17p11.2, is associated with distinctive phenotypic features and mental retardation. Most patients are deleted for the interval between the markers D17S58 and cCI17-498. This interval includes the locus for the human sterol regulatory element binding protein-1 (SREBP-1) gene, which produces isoforms a and c by alternative splicing. The SREBP family of transcription factors transduces intracellular signals activated by cholesterol into the nucleus to induce expression of several genes responsible for the synthesis of cholesterol and LDL-receptors. Past clinical studies have documented hypercholesterolemia, mainly due to an increased LDL fraction, in some children with SMS (Smith et al., 1998, AJHG 63(4) Suppl. A19). To investigate the effects of haploinsufficiency of SREBP-1a and -1c in humans, we performed molecular and cytogenetic studies on fibroblasts from children with documented hypercholesterolemia who were deleted for the SMS critical region.

FISH, performed using purified BAC clones containing nucleotides 368-660 of SREBP-1 cDNA as a probe, demonstrated deletion of SREBP-1 in 10 of 10 children with SMS. On Northern blot analysis, fibroblasts from 3 SMS patients manifested elevated levels of mRNA for HMGCoA reductase, farnesyl diphosphate synthetase and squalene synthetase, enzymes involved in cholesterol synthesis. There was also upregulation of SREBP-2 mRNA.

Several transgenic mouse models exist to study the regulation of cholesterol biosynthesis by SREBPs. In mice homozygously deleted for SREBP-1a and -1c, the levels of SREBP-2 were increased along with increased transcription of genes encoding cholesterol biosynthetic enzymes; heterozygotes appeared normal (Shimano et al., 1997, JCI 100(8): 2115-2124). Nevertheless, we are considering whether haploinsufficiency of SREBP-1 in human fibroblasts might produce effects comparable to those in mice homozygously disrupted for SREBP-1.

Program Nr: 361 from the 1999 ASHG Annual Meeting

Creating a mouse model for DiGeorge(DGS)/Velocardiofacial Syndromes. *P.C. Hsieh[1,2], W. Kimber[3], A. Chen[2], S. Hirotsuni[2], R. Paylor[4], A. Wynshaw-Boris[2,5]*. 1) HHMI Research Scholars Program, HHMI-NIH, Bethesda, MD; 2) Genetic Diseases Research Branch, National Human Genome Research Institute, National Institute of Health, Bethesda, MD; 3) Department of Physiology, School of Medicine, Johns Hopkins University, Baltimore, MD; 4) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 5) Department of Pediatrics, School of Medicine, University of California, San Diego, CA.

DiGeorge syndrome is a clinical disorder caused by failure of third and fourth branchial derivatives to develop properly during embryogenesis. DiGeorge patients typically have impaired cellular immunity, hypocalcemia, congenital heart defects, and facial dysmorphism. Genetic analyses have shown that over 90% of DiGeorge patients have hemizygous interstitial deletions of ~2Mb in human 22q11. Over 85% of Velocardiofacial patients and more than 25% of Conotruncal Face Anomaly patients have similar hemizygous deletions. Therefore, these syndromes are a group of related disorders resulting from haploinsufficiency of gene(s) located in 22q11. Comparative mappings between the human and mouse genomes have shown that syntenic region of 22q11 is conserved on mouse chromosome 16, despite differences in gene order. Using a conventional gene targeting, the DiGeorge Minimal Critical Region (DGMCR) was deleted in ES cells. The ~150kb deletion was confirmed by Pulse-Field Electrophoresis and FISH. Chimeras were generated with this DiGeorge Direct Deletion ES clone, and they were healthy and viable. DiGeorge Direct Deletion (DGDD) mouse lines were generated from these chimeras. Analyses of DGDD mice have shown a marked reduction in fertility in the 129 inbred DGDD heterozygotes compared to wild-type mice. Behavior studies have shown an increase in pre-pulse inhibition in DGDD heterozygous mice. Homozygous DGDD in both backgrounds are early embryonic lethal, indicating gene(s) within DGMCR are essential for embryonic development.

Program Nr: 362 from the 1999 ASHG Annual Meeting

Gastroesophageal reflux and irritable bowel syndrome in classical and hypermobile Ehlers Danlos syndrome (EDS). *H.P. Levy[1], W. Mayoral[2], K. Collier[2], T.L. Tio[2], C.A. Francomano[1]*. 1) NHGRI, NIH, Bethesda, MD; 2) Georgetown University Medical Center, Washington, DC.

Rupture of the GI tract is a known manifestation of vascular EDS (type IV), and diverticuli and hiatal hernia have been reported without specification of EDS subtype. However, the spectrum and frequency of GI complications in classical (types I and II) and hypermobile (type III) EDS have not been established.

We reviewed the charts of 51 EDS patients for GI findings. 38 patients had classical, and 13 had hypermobile EDS. Age range was 8 to 78 years. Sex ratio was biased, with F:M=41:10 (30:8 classical and 11:2 hypermobile). Gastroesophageal reflux (GERD) was suspected clinically in 26 of 45 patients [58%] for whom data was available (17 of 32 classical [53%] and 9 of 13 hypermobile [69%]). Irritable bowel syndrome (IBS), defined as chronic intermittent diarrhea and/or constipation, with or without abdominal cramps, was reported by 24 of 43 patients (56%) for whom data was available (18 of 30 classical [60%] and 6 of 13 hypermobile [46%]. Also, 4 patients (2 classical, 2 hypermobile) had endoscopic diagnoses of non-specific inflammatory bowel disease (IBD); thus 28 of 47 informative patients [60%] had IBS or IBD. Overall, 34 of 47 patients [72%] had any combination of GERD, IBS & IBD. If all missing data was considered negative, the overall prevalence would be 26 of 51 [51%] for GERD and 28 of 51 [55%] for IBS/IBD.

EDS patients may have chronic pain, leading to NSAID-induced gastritis mimicking GERD or narcotic-induced symptoms mimicking IBS. Alternatively, EDS may cause reduced lower esophageal sphincter tone, increased distensibility, and/or decreased GI motility, resulting in GERD and/or IBS. Autonomic dysfunction could cause both GERD and IBS. 10 patients had suspected or confirmed cardiovascular autonomic dysfunction; 9 also had GI complications.

We conclude that GERD and IBS are common complications of classical and hypermobile EDS, and should be sought and treated in these patients.

Program Nr: 363 from the 1999 ASHG Annual Meeting

Clinical features of familial thoracic aortic aneurysms/dissections. *D.M. Milewicz[1], M. Jewell[1], M. Willing[2], D. Guo[1]*. 1) Dept Internal Medicine, Univ Texas Medical Sch, Houston, TX; 2) Univ of Iowa, Iowa City, Iowa.

Autosomal dominant inheritance of thoracic aortic aneurysms and dissections (TAA) occurs in individuals with Marfan syndrome (MFS) and is due to FBN1 mutations. TAAs can also be inherited in an autosomal dominant manner in the absence of skeletal and ocular features of MFS. Ten families with multiple members with TAAs in the absence of ocular and skeletal complications of MFS have been identified and medical records and autopsy reports of affected members reviewed. Individuals at risk for developing TAAs underwent echocardiograms to evaluate the proximal aorta. DNA was collected for linkage analysis. The pattern of inheritance of TAAs was autosomal dominant in these families but the age of onset of aortic disease was highly variable (ranging between 26 and 80 years of age in one family) and there was decreased penetrance of the disorder. The majority of affected individuals presented with asymptomatic aortic root dilatation or acute type I dissections; 1 individual presented with an aneurysm involving the descending thoracic aorta. Histopathology of the aorta showed fragmentation and loss of elastic fibers in the media. The majority of affected males had inguinal hernias. Four of the families had an individual with a cerebral aneurysm who did not have an aortic aortic aneurysm. The phenotype was not linked markers within FBN1 in these families. Described here are the clinical features of autosomal dominant TAA syndrome with decreased penetrance and variable expression that is not due to FBN1 mutations. Cerebral aneurysms and inguinal hernias may be other phenotypic manifestations of the syndrome.

Program Nr: 364 from the 1999 ASHG Annual Meeting

Deletion length correlates with IQ in Williams Syndrome. *C.A. Morris[1], C.B. Mervis[2], B.F. Robinson[2], N. DeSilva[3], P.A. Spallone[4], A.D. Stock[4], X. Lu[5], X. Meng[5], M.T. Keating[5].* 1) Pediatrics, Genetics Division, Univ. Nevada Schl. of Medicine, Las Vegas, NV; 2) Psychology, Univ. of Louisville, Louisville, KY; 3) Univ. Kentucky Schl. of Medicine, Lexington, KY; 4) Pathology, Univ. Nevada Schl. of Medicine, Reno, NV; 5) Howard Hughes Institute, Univ of Utah, Salt Lake City, UT.

Williams Syndrome(WS) is a multisystem disorder that includes dysmorphic facial features, supravalvar aortic stenosis(SVAS), an unusual personality, and unique cognitive profile(WSCP) characterized by strengths in auditory rote memory and language and weakness in visuospatial constructive cognition. WS is caused by a 1.5Mb deletion of chromosome 7q11.23, defined by the interval between markers D7S1778 and D7S489A. We have previously described SVAS kindreds with normal IQ but demonstrating the WSCP, associated with smaller deletions within the region ranging from 83-500kb. We have also shown that individuals with classic WS who have deletions greater than 1.5Mb have lower IQ scores than the WS mean. To determine if smaller deletion size is correlated with better cognitive ability in WS, we studied deletion lengths in 200 individuals with the classic phenotype; only one had a shorter deletion (1Mb), bounded on the telomeric end by genomic clone B270D13. That man had a K-BIT IQ of 95, as compared to the WS mean of 66.27(SD 14.73); he also fit the WSCP. Of the remaining individuals, 12 had IQs between 86 and 97. These data suggest that although a short deletion may contribute to a high level of functioning in a small number of individuals with WS, factors other than deletion size must also be involved.

Program Nr: 365 from the 1999 ASHG Annual Meeting

Extreme anticipation and unusual multisystem phenotype in a family with Spinocerebellar Ataxia Type 7 (SCA7). *S.E. Palmer[1], P. Gunaratne[2], C.S. Richards[2], R.A. Saldivar[3], K.S. Kagan-Hallet[1]*. 1) Depts. Pediatrics or Pathology, Univ Texas Health Sci Ctr, San Antonio, TX; 2) Dept. Molec. & Human Genetics, Baylor College of Medicine, Houston, TX; 3) Dept. Pathology, Santa Rosa Childrens' Hosp., San Antonio, TX.

A 14-month-old child presented in the terminal phase of a progressive encephalopathy. Additional features included chronic hepatomegaly, coarse facies, pigmented retinopathy, and episodic mild metabolic acidosis. Neurodegeneration began in mid-infancy. Mitochondrial, metabolic, and hepatic evaluations were negative except for Fanconi pattern in urine. Muscle histology was nonspecific, possibly due to disuse; CNS scans showed atrophy. Paternal family history revealed recent onset of "cone dystrophy" in the father (age 23), and 10-year history of macular degeneration and ataxia in an aunt (age 20s) without diagnosis; the asymptomatic grandfather (age 60) has a brother who became "crippled and blind" (age 50s) whose daughter has "multiple sclerosis" (age 30s). SCA7 mutation analysis of the proband by PCR assay showed an extremely large expansion (size indeterminate, molecular characterization pending). The father was found to carry an expanded SCA7 allele with 52 repeats. Prenatal diagnosis of two sibling pregnancies showed large expansions similar in size to that of the proband. Autopsy of the proband showed two unusual non-CNS findings: 1) papillary fibroelastosis and ventriculomegaly suggestive of hypertrophic cardiomyopathy; and 2) striking renal tubulopathy with dilated tortuous tubules. Cerebellar histology showed pancellular loss and expected intranuclear inclusions. This family displays the striking anticipation with infancy onset known to occur with paternally-transmitted SCA7 alleles. The additional systemic features in the proband, suggestive of mitochondrial or metabolic disorder, are highly likely to be related to the very large expansion since exhaustive clinical workups were otherwise negative. The presence of heart and kidney disease in this young child, apparently due to a greatly expanded SCA7 allele, may provide futher insight about the molecular pathophysiology of this trinucleotide repeat expansion.

Program Nr: 366 from the 1999 ASHG Annual Meeting

The Neonatal Progeroid (Wiedemann-Rautenstrauch) Syndrome. Report of five new cases and review of the literature. *E.K. Pivnick[1], B. Angle[2], R.A. Kaufman[1], B.D. Hall[3], P. Pitukcheewanont[1], J.H. Hersh[2], J.L. Fowlkes[3], L.P. Sanders[4], J.M. O'Brien[4], G.S. Carroll[1], W.M. Gunther[1], H.G. Morrow[5], G.A. Burghen[1], J.C. Ward[1]*. 1) University of Tennessee, Memphis, TN; 2) University of Loisville, Louisville, KY; 3) University of Kentucky, Lexington, KY; 4) Central Baptist Hospital and Maternal-Fetal Medicine, Lexington, KY; 5) South Memphis Clinic, Memphis, TN.

The Neonatal Progeriod Syndrome (NPS) which is also known as the Wiedemann-Rautenstrauch syndrome is a rare autosomal recessive disorder characterized by generalized lipoatrophy except for fat pads in the suprabuttock areas, hypotrichosis of the scalp hair, eyebrows, and eyelashes, relative macrocephaly, triangular face, natal teeth, and micrognathia. We report 5 new patients who demonstrate phenotypic heterogeneity and who represent the single largest series of NPS reported to date. Two of the patients are from an African-American kindred, which have not been reported previously. The fact that there are 2 pairs of siblings among the 5 patients further establishes that NPS is an autosomal recessive condition. This report also includes a complete review of the previously reported 16 patients and compares them to the 5 new patients. Abnormalities in endocrine and lipid metabolism were found in 3 of our 5 patients. Skeletal findings in 2 of our patients demonstrated some new findings as well as the typical radiological abnormalities previously noted in NPS. It is apparent, based on the 21 cases, that mild to moderate mental retardation is common in NPS. Long term follow-up of patients with NPS should provide more information relative to their ultimate psychomotor development. NPS is usually lethal by 7 months, however, on rare occasions, patients have survived past infancy or the teens. Our three surviving patients range in age from 16-23 months. Variability in the phenotype of NPS is clear from the 16 literature cases and the 5 additional cases reported herein. However, the phenotype remains distinct enough to allow a secure diagnosis.

Program Nr: 367 from the 1999 ASHG Annual Meeting

Distinct neurological profile in Williams syndrome. *B.R. Pober, A.M. Szekely.* Dept Genetics, Yale Univ Sch Medicine, New Haven, CT.

Williams syndrome (WS) is due to a chromosome microdeletion involving multiple genes, several of them demonstrating brain-specific expression. However, neurological problems in WS have received little attention in the medical literature. We performed a full neurological evaluation on 51 WS subjects (32 females and 19 males) ages ranging from 6-49yrs (mean=22yrs). The authors, one of whom is a trained neurologist, conducted a neurological review of systems and systematic examination lasting 45 minutes.

The most striking findings included a variety of abnormalities involving cerebellar dysfunction. More than 50% of subjects had impaired control of eye movements (ocular dysmetria, abnormal saccades and jerky pursuit) while >70% had dysmetria, dyssynergia, dysdiadochokinesis and ataxia. Intriguingly, many subjects who had pronounced difficulty in performing rapid alternating movements demonstrated preserved rhythm tapping skills. Another common neurological abnormality included marked hyperreflexia along with pathological reflexes (70% of cases). Several neurological signs were most prevalent in teenagers and young adults, particularly cerebellar dysfunction and reflex abnormalities. Others, like postural instability, resting tremor and frontal release signs, appeared most frequently in older adults.

Among 5 subjects with MRI documented Chiari malformation I (CM I), 3/5 had symptoms (headache, visual complaints) and/or neurological manifestations (i.e. weakness, atrophy, sensory loss, diminished tendon reflexes of the upper extremities) characteristic of CM I. Close to 20% of the remaining subjects had symptoms and at least one sign indicating that they should be screened for CM I.

Our data suggest that persons with WS show a specific pattern of neurological abnormalities including prominent cerebellar dysfunction and distinct age-related changes. These findings can aid clinical management as well as provide insight into abnormal neurological processes that may result from disturbed function of CNS-specific genes in the WS critical region.

Program Nr: 368 from the 1999 ASHG Annual Meeting

Magnetic Transfer Imaging in Smith-Lemli-Opitz Syndrome. *F.D. Porter[1], N. Richert[2], J. Frank[2], N. Nwokoro[1].* 1) HDB/NICHD, NIH, Bethesda, MD; 2) LDRR/CC, NIH, Bethesda, MD.

The Smith-Lemli-Opitz Syndrome (SLOS) is due to an inborn error of cholesterol biosynthesis. Individuals with SLOS have mutations in the 7-DHC reductase gene. Consequently, they have elevated serum levels of 7-DHC and reduced serum cholesterol levels. Currently treatment of SLOS includes dietary cholesterol supplementation. Although marked improvements in behavior have been noted, it is not clear what the long term effect, if any, dietary cholesterol supplementation will have on the central nervous system. The purpose of this study is to determine whether Magnetic Transfer Imaging (MTI) can provide a quantitative measure of abnormal myelin in SLOS patients. MTI imaging measures the exchange between bound (macromolecular) water and free water in tissues after selective saturation of the bound water fraction with an off resonance radiofrequency pulse. The amount of magnetic transfer, calculated as the MT ratio (MTR) is determined by the equation: MTR=M0-Ms/M0x100 where M0 and Ms represent the signal intensities of images obtained with (Ms) and without (M0) the saturation pulse. MTI exams were performed on 10 SLOS patients (4M:6F; age 0.6-12 yr), 3 healthy pediatric patients (mean age 1.4 yr.) and 4 healthy adults (mean age 37 yr.) using 1.5 T unit. Images were obtained using a T1-weighted pulse sequence with and without a MT saturation pulse. MT images were co-registered to each other, and MTR was determined on a voxel by voxel basis. In SLOS patients, an age-dependent increase in the MTR histogram peak (Hp) and histogram mean (Hm) was observed. The Hp increased from 0.26 (at age 0.6yrs) to 0.34 (at age 10 years); and the Hm increased from 0.24 to 0.33 in the same age group. Linear regression analysis demonstrates a relatively strong correlation (r=0.721) between MTR and age in SLOS group. This is in contrast to normal pediatric patients where maximum MTR values in grey and white matter are observed by 20 months of age. Although no longitudinal serial data is yet available, these results suggest that there are ongoing changes in the myelin structure in SLOS patient and this technique may provide a tool for monitoring therapeutic interventions.

Program Nr: 369 from the 1999 ASHG Annual Meeting

Smith-Magenis syndrome: A genomic disorder with a common molecular mechanism and variable clinical phenotype. *L. Potocki[1,3], K.-S. Chen[1], D.E. Osterholm[1], L.G. Shaffer[1], J.R. Lupski[1,2,3]*. 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Pediatrics, Baylor College of Medicine, Houston, TX; 3) Texas Children's Hospital, Houston.

Smith-Magenis syndrome (SMS) is a multiple congenital anomalies mental retardation syndrome associated with a deletion of chromosome 17p11.2. We have previously described the clinical phenotype of SMS in 27 patients (Greenberg et al. 1996). We have extensively characterized the clinical phenotype in an additional 29 SMS patients under the SMS clinical research protocol through the General Clinical Research Center at Texas Children's Hospital. The multidisciplinary study includes developmental and speech and language assessments, ophthalmologic, audiologic, and otolaryngologic evaluation, echocardiogram and EKG, sleep study with EEG, renal ultrasound, scoliosis survey, urinalysis, thyroid function studies, immunoglobulin levels, lipid profile, complete blood count, and levels of blood urea nitrogen and creatinine. Common findings include ophthalmologic anomalies, hearing impairment, cardiac anomalies, hypercholesterolemia, and sleep disturbance. While common phenotypic features can be defined, a wide range of clinical variability exists between patients. It had been postulated that this phenotypic variation among SMS patients could be due to variations in the size of the deletion, although a common deletion region was assigned by polymorphic marker analysis. We have shown that the SMS microdeletion occurs by unequal crossing over due to homologous recombination between flanking repeat gene clusters (SMS-REPs). Herein we present clinical data on 56 SMS patients, and the data of pulsed-field gel analyses on the majority of SMS patients subjected to the multidisciplinary clinical evaluations. A patient-specific junction fragment was identified in more than 90% of SMS patients with the common deletion (as defined by G-band and FISH analyses). Interestingly, although the majority of SMS patients have the same sized deletion, the clinical spectrum is variable. These data suggest that other factors, such as the unmasking of recessive alleles, or modifier loci may cause variability in the phenotype of SMS.

Program Nr: 370 from the 1999 ASHG Annual Meeting

VACTERL-H features associated with a deletion of *ZIC3* in Xq26.2. *S.M. Purandare[1], D. Chitayat[2], G.B. Ferrero[3], C.D. Kashork[4], B. Marino[3], L.G. Shaffer[4], B. Casey[1]*. 1) Pathology, Baylor College of Medicine, Houston, TX; 2) Hospital for Sick Children, University of Toronto, Ontario, Canada; 3) Pediatrics, University of Turin, Turin, Italy; 4) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Loss of appropriate left-right positional information during development results in situs abnormalities. These comprise complete reversal (situs inversus) or indeterminate sidedness (situs ambiguus) of organs in the chest and abdomen. Previously we identified coding-region mutations in an X-linked transcription factor, *ZIC3*, among cases of familial and sporadic situs abnormalities. We now describe a 1.3 Mb deletion encompassing *ZIC3* that segregates through a family with at least four affected males with situs abnormalities and one male with features of VACTERL-H association. Fetal ultrasound and post-mortem analysis of the proband showed situs ambiguus (complex heart malformation, asplenia, right sided stomach and pancreas, bilateral trilobed lungs), renal hyperplasia, imperforate anus, and digit anomalies. His maternal uncle, however, manifested features of VACTERL-H, including dysraphic lumbar spine, imperforate anus, heart malformations, horseshoe kidney, limb anomalies, and aqueductal stenosis with hydrocephalus; no features diagnostic of left-right axis anomalies were present. Sequence-tagged site (STS) analysis of the proband by PCR failed to amplify seven markers between sWXD1184 and DXS1062 inclusively, which span aproximately 1.3 Mb in Xq26.2. FISH analysis using a cosmid probe from the deleted region detected 6 female carriers across 4 generations. A number of VACTERL-H families consistent with X-linked inheritance have been described. Our results raise the possibility that mutations in *ZIC3* and/or closely-linked genes in Xq26.2 may be responsible for some of these cases.

Program Nr: 371 from the 1999 ASHG Annual Meeting

Brachydactyly with fibular hypoplasia is associated with a dominant mutation in CDMP1. *N.H. Robin[1], D.B. Everman[1], J. Hecht[2], S.M. Morrison[3], M.L. Warman[1].* 1) Dept Genetics, Case Western Reserve Univ School of Medicine, Cleveland, OH; 2) University of Texas Medical School, Houston; 3) Dept Radiology, Case Western Reserve Univ School of Medicine, Cleveland, OH.

Brachydactyly (BD) refers to the underdevelopment of skeletal elements within hands and feet. Isolated heritable brachydactylies can be classified into subtypes BDA through BDE based upon the pattern of digital involvement. We have previously shown that one autosomal dominant brachydactyly, BDC, is caused by functional haplo-insufficiency for Cartilage Derived Morphogenetic Protein-1 (CDMP1) on human chromosome 20. Homozygous mutations in this gene also cause two severe skeletal phenotypes, Hunter-Thompson acromesomelic dysplasia (HT) and Grebe syndrome (GS). Each, in addition to hand involvement, has severe reduction defects of the arms and legs; as expected, brachydactyly was observed in obligate heterozygotes in Grebe syndrome families. To determine whether there may exist mutations within CDMP1 which cause phenotypes intermediate between BDC and GS/HT, we evaluated two unrelated probands who manifest brachydactyly and fibular hypoplasia. Fibular hypoplasia has not been observed in BDC patients with previously identified functional haploinsufficiency mutations in CDMP1. Both probands were found to have an identical C-T transition within CDMP1 that changes and Arginine to Tryptophan within the consensus RXXR polypeptide cleavage site. This change was not observed in 100 control alleles. The RXXR cleavage site is necessary for normal processing of CDMP1 protein dimers. One of our two probands has an identically affected sister who also has this mutation, as does her father. However, he manifests only brachydactyly. Our other proband's mother and maternal aunt are reported to have small hands and clubfeet. In addition, this proband's fetus has fibular hypoplasia detected by prenatal ultrasound. DNA and radiographic studies are pending for the mother, aunt, and fetus. Our results suggest that mutations which affect the RXXR cleavage site cause a dominant negative effect leading to a phenotype which is intermediate between BDC and GS/HT.

Program Nr: 372 from the 1999 ASHG Annual Meeting

Frequency and type of mutations within the human JAG1 gene in Alagille Syndrome. *A. Roepke, A. Kujat, M. Graeber, I. Hansmann, I. Giannakudis.* Humangenetik, Martin-Luther-Universitt, Halle/S., Sachsen-Anhalt, Germany.

Alagille syndrome (AGS, MIM# 118450, McKusick, 1998) is an autosomal dominant disorder characterized by liver disease with heart, skeletal, ocular and facial abnormalities. The gene JAG1 on chromosom 20p12 has been identified as responsible for AGS. JAG1 encodes a ligand in the Notch signaling pathway, that plays an important role in cell fate determination. JAG1 spans 36 kb, comprises 26 exons and encodes a 1218 amino acid protein with several domains and high interspecies conservations. We screened 74 AGS patients by SSCP and sequencing to evaluate frequency and type of mutation in the JAG1 gene. Of the 74 patients, 50 (68%) have mutations within JAG1: we found 13 (18%) small deletions, 6 (8%) small insertions, 10 (14%) nonsense mutations, 9 (12%) missense mutations and 12 (16%) splice site mutations. Most of the mutations (88%) are, by computational analysis, expected to result in a truncated protein thus leading to loss of functional domains that are responsible for the interaction with the Notch receptor. This observation is consistent with haploinsufficiency of JAG1 as well as with a dominant negative effect. One mutation was found in exon 26 within the transmembrane domain. This mutation should reduce the hydrophobicity of this region. 8 of 9 missense mutations occur in exons 4-6 and show an exchange of amino acids which are highly conserved from Drosophila to human. These regions may be important for the ligand-receptor interactions. Yet, the mutations are distributed over the entire gene evidence is provided for a mutational bias in exon 5 and 17. To date no mutations were found in exon 8. In 25 out of 41 cases (61%) a de novo mutation was found. In 16 families (39%) the mutation could be determined as maternal (n=10) or paternal (n=6). According to our analysis which includes deletion screening by microsatellites as well, up to 10% of the cases are due to genetic mosaicism. Mosaicism was also detected in clinically normal patients with AGS, which has to be taken into account in genetic counselling. This work was supported by the DFG.

Program Nr: 373 from the 1999 ASHG Annual Meeting

The Neurodevelopmental Profile of Young Children with Sex Chromosome Variations (SCV)Prenatally Diagnosed. *C.A. Samango-Sprouse*[1,2]. 1) Pediatrics, Children's Natl Medical Ctr, Washington, DC; 2) George Washington University Washington, D.C. 20020.

Few studies have investigated the neurocognitive profile of young children with SCV who are prenatally diagnosed. A large prospective study of 43 subjects with SCV was undertaken.The Bayley Scales of Infant Development(BSID-II), the Gesell Developmental Schedules and the Preschool Language Scale(PLS-3) were used for the neurodevelopmental assessments. Socioeconomic status was determined using the Hollingshead Social Class Index. Muscle tonus was evaluated using the Bobath neurodevelopmental approach. Data was compared to population norms since family studies were not possible because many probands were often first children. The mean age of the study subjects was 16 months (range 2-60 mos). There were 35 XXY, 3 XXX and 4 XYY. 36 patients were Caucasian, 2 were African-American and 3 were of Hispanic origin.The social class was class 3 or middle income or higher.The mean MDI was 99.70 (sd=20.5)with several children testing in the superior range of intelligence. The mean PDI ws 100.3(sd=18.5),the mean fine-motor score(FMDI) was 102.58, the mean receptive language score (RL)was 98.76(sd=18.9),and the mean expressive language score(El)was 89.5 (sd=16.5). Findings from the BSID indicate high risk factors in attention and emotional regulation. 20 percent of children had torticollis and significant delays in unilateral balance reactions. Truncal hypotonia was present in 68 percent and 40 percent had appendicular hypotonia. There was a significant discrepancy between expressive and receptive language skills that was evident by 18 months of age. 33 percent of all children received early intervention services. Preliminary findings of this study demonstrate an increased prevalence of developmental disruption in children with SCV evident during preschool years. Further studies are necessary to determine the natural history of brain and behavior relationships associated with SCV. Families should be counseled that these children may require therapeutic services during preschool years to optimize their neurocognitive development and school achievement.

Program Nr: 374 from the 1999 ASHG Annual Meeting

Recognition of Congenital Heart Disease as a component of a distinct Prader-Willi Syndrome infant phenotype due to a combination of Mosaic Trisomy 15 with Maternal Uniparental Disomy 15. *L.R. Shapiro[1,3], M. Gewitz[2,3], P. Woolf[2,3], B. Fish[2,3], S. Ingram[1], B.F. Golden[1], R. Marion[4].* 1) Pediatrics/Medical Genetics, New York Medical Col, Valhalla, NY; 2) Pediatrics/Cardiology, New York Medical Col, Valhalla, NY; 3) Westchester Medical Ctr, Valhalla, NY; 4) Albert Einstein Col of Medicine, Bronx, NY.

The usual infant phenotype of Prader-Willi Syndrome (PWS) includes hypotonia and craniofacial features but *not* congenital heart disease (CHD). The major pathogenic mechanisms are a deletion of a paternal chromosome 15(q11-q13) or maternal uniparental disomy (UPD) of chromosome 15 resulting from maternal meiotic non-disjunction, subsequent trisomy 15 and *rescue* by loss of the paternal chromosome 15 in some cells (trisomy 15 mosaic) or all cells.

While trisomy 15 mosaicism has been reported in amniocytes and CVS, only 4 liveborn infants have been reported and had severe CHD, facial dysmorphism and skeletal abnormalities. The *combination* of trisomy 15 mosaicism *with* maternal UPD 15 has been reported in only 4 liveborns who had hypotonia (4/4), dysmorphic facies (3/4) and CHD (2/4); however, the diagnosis of PWS was not considered in any of these infants. When trisomy 15 mosaicism *combined* with maternal UPD 15 was detected prenatally, it could be confirmed postnatally in fibroblasts but not lymphocytes.

Two*additional* liveborn infants are hereby reported who were diagnosed with PWS (hypotonia and facial features) *and* severe CHD and found to have the combination of low level trisomy 15 mosaicism *with* maternal UPD 15. With recognition and approriate diagnostic testing, it is possible that more infants with severe CHD will be found to have the *combination* of low level trisomy 15 mosaicism with maternal UPD 15.

In conclusion, severe CHD in an infant with hypotonia and facial dysmorphism may be due to this distinct form of PWS which may represent a more common cause of severe CHD in early infancy than previously appreciated.

Program Nr: 375 from the 1999 ASHG Annual Meeting

Loss of chromosome 13 and tetraploidy in human vascular endothelial cells in culture. *H.M. Aviv[1], L. Zhang[2], S. Patel[1], A. Bardeguez[3], K. Okuda[2], M. Kimura[2], A. Aviv[2].* 1) Center for Human and Molec. Genetics; 2) Hypetension Res. Center; 3) Dept. of OB/GYN, UMDNJ, Newark, NJ.

Previously, we found that *in vivo* the frequency of aneuploidy, mainly tetrasomy, of vascular endothelial cells (VECs) of the human abdominal aorta increased with the donor's age, reaching a prevalence of 30% of VECs in older persons. In this study, we monitored aneuploidy in serially passed VECs from the umbilical veins of 8 healthy newborns until replicative senescence. The cells were cultured in enriched growth medium containing a number of growth factors. Chromosome analysis was performed approximately every 7 population doublings (PD), continuing up to a few PDs before senescence. Chromosome analysis was also performed in 3 fibroblast cell strains from circumcision skin specimens of newborns.

Initially, all cultures of VECs had normal karyotypes. However, a consistent pattern of aneuploidy emerged in later passages. Seven of the 8 cell strains lost chromosome 13 in 100% of metaphase cells in mid-passage (PD 28-56) and 5 of the 8 cell strains gained chromosome 11, although usually not in all metaphase cells. Only cell strain #2, the cell strain that did not exhibit loss of chromosome 13, gained chromosome 11 in 100% of cells by PD 28. Five of the 7 cell strains that lost chromosome 13 in 100% of cells became hypotetraploid by the next chromosome analysis. The fibroblast cell strains, grown until senescence in enriched and regular growth medium did not exhibit consistent aneuploidy. Thus, it appears that loss of chromosome 13 is a constant feature of human VECs. Interestingly, this chromosome harbors the Rb and the endothelin-B receptor genes. The former is a mitotic checkpoint gene while the latter is a specific growth regulator of VECs. Moreover, the loss of chromosome 13 triggers the development of tetraploidy of VECs *in vitro*. Our findings are in line with the concept that the genetic make-up of VECs changes both *in vitro* (as a function of cell replication) and *in vivo* (as a function of the donor's age). These results have broad implications to the development of complex genetic traits, which may be triggered by altered gene expression as human beings age.

Program Nr: 376 from the 1999 ASHG Annual Meeting

Cell cycle-dependent expression and subcellular localization of human barren-1 (HCAP-H). *O.A. Cabello[1], E. Eliseeva[3], I. Ouspenski[1], I. Coldwell[4], F. Herbert[1], S. Thang[1], C.A. Austin[4], B.R. Brinkley[1], S. Plon[3,2], J.W. Belmont[2,3].*
1) Dept Cell Biol, Baylor Col Med, Houston, TX; 2) Dept Mol & Human Gen, Baylor Col Med, Houston, TX; 3) Dept Pediatrics, Baylor Col Med, Houston, TX; 4) Dept Biochem & Genetics, U. New Castle Upon Tyne, U.K.

Drosophila *barren* is required for chromatid arm separation. Its homolog in Xenopus, XCAP-H, is a subunit of the conserved heteropentamer 13S condensin and is required for mitotic chromosome condensation. HCAP-H fully complements the growth arrest phenotype of yeast mutant *brn1* and of a temperature-sensitive *brn1* allele, indicating that *BRN1* and HCAP-H are functional orthologs. However, in contrast to previous reports, HCAP-H does not interact with topoisomerase II a or b, as indicated by GST pull-down, co-immunoprecipitation, and yeast two-hybrid interaction assays, nor does recombinant HCAP-H modify the decatenation activity of topoisomerase II a in vitro. We have previously mapped the human brrn-1/HCAP-H locus to 2q11.2. Northern blot analyses of PHA-stimulated lymphocytes and Molt-4 elutriated cells, indicated that HCAP-H is transcribed during G2. Although the HCAP-H mRNA level is cell cycle-dependent, Western blots with two affinity-purified antibodies indicate that HCAP-H protein levels remain stable throughout the cell cycle in HeLa cells. These results suggest that the mitotic function of HCAP-H may require newly synthesized protein and that HCAP-H might fulfill a second function during interphase. Immunofluorescence microscopy of HeLa cells demonstrated that the subcellular localization of HCAP-H is cell cycle regulated: during mitosis it associates with discrete regions along condensed chromosomes; during interphase, it exhibits a distinct punctate nucleolar distribution. The staining pattern is specific, as demonstrated by epitope competition. These results are consistent with a role of HCAP-H in mitotic chromosome condensation. Moreover, we propose that HCAP-H may fulfill a second role in interphase nucleoli, possibly modulating the conformation and function of rDNA.

Program Nr: 377 from the 1999 ASHG Annual Meeting

The concept of chromosome condensation and de-condensation during mitosis has to be replaced by the concept of chromosome region specific protein swelling. *U. Claussen[1], J. Lemke[1], S. Michel[1], I. Chudoba[1], P. Muehlig[2], M. Westermann[3], J. Claussen[1], N. Rubtsov[4], U.W. Grummt[5]*. 1) Inst Human Gen & Anthropology, Jena, Germany; 2) Molecular Biotechnology, Jena, Germany; 3) Ultrastructural Research, Jena, Germany; 4) Cytology and Genetics, Novosibirsk, Russia; 5) Physical Chemistry, Jena, Germany.

The concept of chromosome condensation and de-condensation during mitosis has been investigated. The morphological changes of mitotic cells and chromosomes were analyzed during treatment with solutions needed for chromosome preparation such as fixative, acetic acid, and mixtures of water and acetic acid. Furthermore, chromosome stretching experiments were performed to analyze the splitting process of Giemsa bands into their sub-bands. Surprisingly, chromosome spreading is composed of a dramatic water-induced swelling of mitosis prior to evaporation of the fixative and subsequent flattening. Even singel chromosomes swell which leads to an elongation up to two times the original length. Chromosome swelling preferentially takes place in Giemsa light bands. Prophase, prometaphase and metaphase chromosomes are therefore preparation induced reproducible artifacts. The biology behind the chromosome region-specific swelling is not well understood. The mitotic and chromosome swelling process is a gel-like one and is a prerequisite for metaphase spreading, for the formation of the G-banding pattern, and for the production of cytogenetically analyzable chromosomes. Consequently, the concept of chromosome condensation and de-condensation during mitosis should be replaced by the concept of chromosome region specific protein swelling. Chromosome 5 specific high resolution multicolor-banding (MCB) experiments confirm that at the DNA level chromosomes in interphase nuclei show the same banding pattern as those in late metaphase and therefore are not de-condensed. The DNA specific chromosome banding pattern in interphase has even been used for the detection of single chromosome band deletions which opens up new fields in molecular cytogenetics.

Program Nr: 378 from the 1999 ASHG Annual Meeting

Systematic mapping of balanced chromosome rearrangements in association with Russell-Silver syndrome (RSS). *S. Doerr[1], M.L. Ayala-Madrigal[1,2], A.T. Midro[3], I. Giannakudis[1], I. Hansmann[1].* 1) Inst. f. Humangenetik, Halle, Germany; 2) UdeG and CIBO, IMSS Guad., Mexico; 3) Dept. of Clin. Genetics, Bialystok, Poland.

Russell-Silver syndrome is a heterogeneous disorder mainly characterized by pre- and postnatal growth retardation and some morphological abnormalities. Most cases are sporadic although in a minority familial inheritance has been described. About 10 % of patients show maternal uniparental disomy of chromosome 7. This is in good agreement with the data of our own survey identifying 3 cases of maternal UPD7 in a group of 31 probands. Furthermore, two autosomal translocations involving band 17q25 were reported in association with RSS (Ramirez-Dueñas et. al., 1992; Midro et. al., 1993). Molecular analysis of the breakpoints on chromosome 17 enables us to refine the localization of the breakpoint of the de novo translocation previously described as t(1;17)(q31;q25) to 17q23-24, whereas the breakpoint of the translocation described as t(17;20)(q25;q13) is localized in 17q25. Since the cytogenetic and molecular analysis of disease-associated balanced chromosome rearrangements is an efficient strategy to map and clone disease genes we cloned the breakpoints of the 1;17 translocation, and cloning of the 17;20 translocation is in progress. In order to identify a gene for RSS in the region 17q23-24 a YAC/PAC/cosmid contig (~ 5 Mb) for the RSS critical region around the breakpoint was constructed. This contig comprises loci for 30 STSs and 13 genes/ESTs. By searching for expressed sequences within the breakpoint area we identified a gene of a multigene family in close proximity to the breakpoint. Interestingly another member of this family is located in 17q25 close to the breakpoint segment of the second translocation. As a prerequisition for mutation screening in 31 probands with RSS, we determined the genomic structure of the gene within 17q23-24. Searching for mutations is in progress by direct sequencing of the identified 11 exons. Ongoing analysis led to the identification of 6 intragenic polymorphisms representing 4 common haplotypes in controls, but only 2 haplotypes exist in probands. Whether these observations contribute to the etiology of the disease is under investigation.

Program Nr: 379 from the 1999 ASHG Annual Meeting

Chromosomal Study of 300 Children born after Intracytoplasmic Sperm Injection(ICSI). *E.S. Elsobky[1], M.A. Aboulghar[2], R.T. Mansour[2], G.I. Serour[2].* 1) Human Genetics Ctr, Heliopolis, Cairo, Egypt; 2) Egyptian IVF-ET Ctr, Cairo,Egypt.

Conventional in-vitro fertilization (IVF) may be inadequate to allow fertilization in cases of male factor infertility, Intracytoplasmic sperm injection into the egg(ICSI;Palermo et al, 1992)have remarkably changed the opportunity to achieve pregnancy in such cases, yet it is an invasive procedure using in many cases a critically poor sperm quality bypassing natural selective mechanisms against the disomic gametes with potential cytogenetic risk. To study the cytogenetic impact of ICSI on its outcome we performed blood chromosomes for 300 children born after ICSI.Chromosomal abnormalities were identified in 13 cases(4.3%)including:Sex chromosome abnormalities in 7 cases(2.3%),Autosomal aneuploidy(Trisomies)in 3 cases(1%),Structural abnormalities(reciprocal translation)in 3 cases(1%)and rare case with three associated abnormalities(sex chromosome aneuploidy,autosomal inversion and reciprocal translocation).These results reflect the high incidence of chromosome abnormalities among children born after ICSI(almost 7 times the population incidence),this could be attributed to two possible major roots;increased incidence of sex chromosome abnormalities due to meiotic errors in subfertile males with high frequency of disomic sperms, and direct transmission of paternal structural chromosomal abnormalities which is also high among infertile men treated rather than ICSI procedure.

Program Nr: 380 from the 1999 ASHG Annual Meeting

Higher frequencies of X-Y aneuploid sperm in fathers of boys with Klinefelter syndrome when the extra X-chromosome was of paternal origin. *B. Eskenazi[1], X. Lowe[2], S. Kidd[1], K. Weisiger[1], D. Moore II[2], Y-J. Chuu[2], M. Aylstock[3], A. Wyrobek[2]*. 1) School of Public Health, University of California, Berkeley, CA; 2) Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, CA; 3) Klinefelter Syndrome and Associates, Roseville, CA.

The objective of this research was to determine whether men with elevated frequencies of disomic sperm are at greater risk of fathering trisomic children. In 36 families who had a boy with Klinefelter syndrome (KS; 47,XXY; <6yrs old), inheritance of the extra X-chromosome was paternal in 10 and maternal in 26 families, based on 4 polymorphic microsatellite markers. Frequencies of sperm with aneuploidies involving chromosomes X, Y, or 21 were examined for all fathers by four-color sperm FISH (10,000 sperm per man), to determine whether fathers of the paternally-inherited KS group produced higher frequencies of aneuploid sperm than did the fathers of the maternally-inherited KS group. Specific classes of sperm aneuploidy were evaluated: X-Y sperm (which might produce KS upon fertilization), 21-21 (for Down syndrome), as well as X-X or Y-Y (Meiosis II errors). Fathers of the paternal KS group showed higher frequencies of X-Y sperm (median, 13.5 vs. 8.0 per 10K; p=0.01, crude; p=0.16, adjusted for age) and total hyperhaploidy (sum of hyperhaploidy for X, Y and 21) (median, 36.5 vs. 23.5; p=0.03, crude; p=.15, adjusted for age). Men who were older (at least 40 years) and had a boy with paternally-inherited KS had the highest rates of X-Y aneuploidy. Frequencies of X-Y and 21-21 sperm were correlated among men (Pearson's r=0.42, p=0.01), suggesting that trans-chromosomal mechanisms may be involved. However, frequencies of sperm with X-X, Y-Y, and 21-21 disomies did not differ between the KS groups (p>0.2). These findings support the hypothesis that men with higher rates of aneuploidy in their sperm are more likely to father children with trisomy.[Supported by NIEHS Superfund grant P4ZES04705 to UCB with subcontact to LLNL; partially performed under the auspices of the U.S. DOE by LLNL under contract W-7405-ENG-48.].

Program Nr: 381 from the 1999 ASHG Annual Meeting

Sex chromosome mosaicism originating at the myeloid/lymphoid cell lineage. *W.L. Flejter[1], E. Jackle[1], S. Aufox[1], K. Geisinger[2], M.J. Pettenati[1]*. 1) Pediatrics/Medical Genetics; 2) Pathology, Wake Forest Univ Sch Med, Winston-Salem, NC.

A peripheral blood sample was received for Fragile X testing. DNA analysis was negative, although the results suggested the presence of two X chromosomes. FISH analysis of the unstimulated blood, using X and Y chromosome probes, revealed four cell lines; XY/XXY/ XYY/ XXYY. A second blood sample was obtained for further studies. Conventional chromosome analysis of PHA-stimulated blood showed only 48,XXYY cells. Interphase FISH studies on the second sample, after PHA stimulation for 48, 72 and 96 hrs, again showed multiple sex chromosome cell lines. Interestingly, the frequency of XXYY cells increased over time in culture while the frequency of the other cell lines decreased. Subsequently, a buccal smear was obtained to see if mosaicism was present in a different cell type. FISH results showed XY, XXY and XXYY cell lines. To determine if discordance between the chromosome and FISH analyses could be explained on the basis of cell lineage, cytological staining of the slide preparations was performed. FISH results clearly showed that lymphoid cells had only an XXYY chromosome constitution while the myeloid cells showed all four cell lines.

Discordant karyotypes have been described as a relatively frequent finding in different tissue types from the same patient. However, discordant results in stimulated vs. unstimulated cultures have not been well documented. One hypothesis for the discrepancy is that normal cells are at a growth advantage over abnormal cells in culture. However, our results, as well as other published studies, suggest that metaphase cells found at direct analysis represent a different population of cells than those present after PHA-stimulation. These findings raise important questions regarding accurate karyotype results particularly in cases of prenatal diagnosis after abnormal ultrasound, a normal karyotype in a dysmorphic child, suspected mosaicism in individuals who don't fit the characteristic phenotype of a distinct genetic syndrome, and in patients with recurring pregnancy loss or infertility.

Program Nr: 382 from the 1999 ASHG Annual Meeting

Delayed DNA replication leads to chromosomal instability at FRA7H, a common fragile site on human chromosom 7. *A. Hellman[1], A. Rahat[1], S.W. Scherer[2], L.C. Kerem[2], B.[1].* 1) Department of Genetics, The life sciences Institute, The hebrew University, Jerusalem, Israel; 2) Department of Genetics, The hospital for sick Children, Ontario, Canada.

Common fragile sites are specific unstable chromosomal loci characterised by constrictions, gaps or breaks on chromosomes from cells exposed to inhibition of DNA replication. In contrast to the rare fragile sites, no expanded repeats have been found in the common fragile sites, and the molecular basis for their fragility is currently unknown. Here we studied the replication pattern of FRA7H, a common fragile site on human chromosome 7. Using a FISH based method we found that ~30% of S-phase nuclei from nonsynchronyzed cell culture showed two unreplicated alleles (SS signals), indicating that the replication of FRA7H is initiated relatively early in the S phase. However, a high fraction, 35%, of the S-phase nuclei showed one replicated and one unreplicated allele (SD signals), indicating that a considerable fraction of FRA7H alleles accomplished its replication only during late-S phase. This allelic asynchrony is not due to allele specific replication time as found in the analysis of a chromosome 7 isodysomic and a marked chromosome 7 cell lines. The cytogenetic expression of FRA7H occurs over a region of ~160 kb. Upon aphidicolin treatment (the FRA7H inducer and an inhibitor of DNA synthesis), large difference appeared between the replication time of the FRA7H central region (D=44%) and both of its sides (D=19%). Two-colour FISH analysis using pairs of adjacent clones from the FRA7H region revealed that in a large fraction (15-25%) of the S-phase nuclei the central FRA7H region accomplished its replication while the adjacent sequences from both sides did not. All these results led us to propose that the fragility of FRA7H is a consequence of a low rate of DNA synthesis, along a single replicon comprising this fragile region. This low rate might result from random formation of non-B DNA structures, which interfere with the replication elongation. Upon aphidicolin treatment, some FRA7H alleles may fail to overcome this interference, resulting in the fragile site expression.

Program Nr: 383 from the 1999 ASHG Annual Meeting

Microdeletion at 12q24.1 within the NS critical interval in two unrelated patients presenting with Noonan syndrome. *A.M. Ion, A. Brady, A. Crosby, K. Marks, S. Jeffery, M.A. Patton.* Medical Genetics, St George's Hospital, London, UK.

Noonan Syndrome (NS) is a genetic condition characterised by a typical facial appearance, "Turner-like" phenotype and congenital heart defects, particularly pulmonary valve stenosis and hypertrophic cardiomyopathy. This disorder has been previously mapped at the chromosomal band 12q24.1 to a 10 Mb interval between markers D12S84 and D12S79. As part of our strategy for cloning and characterising genes involved in this condition we screened several patients presenting with typical Noonan syndrome and learning disability for submicroscopic chromosomal abnormalities. FISH probes included several cosmids that result from the subcloning of two YACs spanning part of the NS critical interval. We report here two patients presenting with typical NS and abnormal hybridisation pattern for one or several of the cosmids mentioned above. FISH performed on metaphase spreads from these patients identified four cosmids that were partially or completely deleted in the two patients. The four cosmids were subcloned from the same YAC that spans an interval of approximately 1.4 Mb of the NS critical rgion. Results were confirmed in both patients by several successive FISH techniques (over 50 metaphase spreads). Molecular analysis in the first patient showed a missing allele for one polymorphic marker within the 1.4 Mb interval. However, the same marker was not informative for the second patient. Both patients were heterozygous for all other informative markers in the region. This findings suggest a microdeletion present in both patients that may be associated to the Noonan syndrome phenotype.

Program Nr: 384 from the 1999 ASHG Annual Meeting

Development and validation of exon-specific FISH probes for DMD carrier detection. *M. Jaju[1], A.H. Ligon[2], C.S. Richards[1], C.D. Kashork[1], L.G. Shaffer[1].* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Departments of Obstetrics /Gynecology & Pathology, Brigham & Women's Hospital/Harvard Medical School, Boston, MA.

Duchenne Muscular Dystrophy (DMD) is an X-linked disorder affecting about 1/3500 liveborn males. Approximately 65% of mutations in the dystrophin gene are deletions. Current molecular methods of carrier detection in females depend on dosage assessment. We report the use of FISH as an alternative method for deletion detection. To date, we have identified 13 exon-specific cosmids that have been used for FISH. The current probe set includes exons 3-6, 8, 12, 13, 17, 19, 43-45, 48, 50, 51, and 60. The validity of these probes for providing direct detection of dystrophin exon deletions and establishing carrier status was demonstrated by comparing FISH results with standard molecular diagnosis in three groups of families: (1) Xp21 contiguous gene deletion (CGD) probands and their female relatives (n=8); (2) mothers/female relatives and corresponding DMD-affected males (n=6); and (3) mothers/female relatives for whom no corresponding affected male was available for study (n=14). Results of FISH and molecular analyses were available for 11 of 14 affected males studied. In every case, the expected deletion was confirmed using FISH. In three CGD probands for whom molecular analysis was unavailable, deletions were demonstrated using multiple FISH probes. For these 14 families, 7 of 15 female relatives studied were identified as deletion carriers using the family-specific FISH probes. These FISH results were 100% concordant with available molecular results. In the 14 families for whom affected males were unavailable, deletions were detected in 5 of 16 females tested. Of these, molecular diagnosis had been performed in 14 cases and the FISH results were concordant in every case. Our study demonstrates that FISH is an accurate and reliable approach to identifying carriers of DMD deletions.

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Terminal deletions may not be terminal: FISHing for mechanisms of chromosomal 1p36 deletions using telomere region-specific probes. *C.D. Kashork, B.C. Ballif, S.K. Shapira, L.G. Shaffer.* Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Chromosome integrity is dependent upon the presence of telomeric sequences. Thus, cytogenetically defined terminal deletions must have telomeres to be structurally stable. Three possibilities for apparent, terminal deletions exist: true terminal deletions that stabilize through a process of telomere replacement or acquisition, interstitial deletions that retain the original telomere, and derivative chromosomes which obtain a different telomere through cytogenetic rearrangement. We report the use of telomere region-specific probes to characterize apparent, cytogenetic terminal deletions in patients with 1p36 monosomy. The probes consist of sub-telomeric DNA sequences unique to each chromosome arm (Cytocell). Simultaneous FISH for 41 telomeric regions was performed on 32 patients with 1p36 deletions. All patients were previously studied using cytogenetic and molecular methods, and were found to have varying sized deletions. Results using the telomere region-specific probes showed 5 patients had *de novo* telomeric rearrangements, including 3 cases of derivative chromosomes 1p with 1q sequences identified, one case with 2p telomeric sequences on 1p, and one case with Xp sequences replacing the 1p. An additional case was previously known to have a satellited 1p, not detectable by this assay. 21 patients had apparent terminal deletions of 1p with no other telomeric rearrangement detected, and 5 patients showed no deletion of the 1p probe, indicating interstitial deletions. Of the 5 patients with interstitial deletions, 2 were previously not known, but uncovered with the use of the 1p probe. For 6 cases, regions of homology near the 1p telomere may have allowed for recombination to occur with non-homologous segments, leading to derivative chromosomes. Of the 21 terminal deletions, the chromosomes must have been stabilized through the addition of $(TTAGGG)_n$ sequences. The mechanism of how these deletions acquired the necessary terminal cap is under investigation. Our findings may help delineate mechanisms for other terminal deletions, in addition to those involved in monosomy 1p36.

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The variant smaller DGS/VCFS deletions occur at two distinct blocks of duplicated sequence within the 3 Mb common deletion. *H. Kurahashi, T. Shaikh, S. Saitta, B. Emanuel.* The Childrens Hospital of Philadelphia, Phila.,PA.

The 22q11.2 deletions associated with DGS/VCFS occur with a high frequency. It has been suggested that they result from recombination involving duplicated sequence blocks (duplicons) interspersed in 22q11.2. The deletion interval contains four duplicons which are repeated at several other locations on 22q. The deletion endpoints (DEPs) appear to occur within the duplicons in regions we have designated A, B, C, and D. The A-D 3 Mb deletion occurs most frequently. Further, we and others have previously described several patients with atypical deletions (A-B, A-C, C-D). To determine the organization of the duplicons and their role in deletion formation, we have constructed a contig across the four DEP regions using BAC, PAC and cosmids. The size of each duplicon is greater than 100 kb. Whereas A and D contain at least two copies of the duplicon, B and C appear to contain a single copy. By sequence analysis the duplicons share greater than 95% identity and each of them contains common elements. To further examine the role of the duplicons in deletion formation, we have performed pulsed field gel electrophoresis of genomic DNA from A-B and A-C deletions. Southern hybridization with probes flanking the deleted region have identified novel rearrangement fragments. For A-B deletions we used a probe immediately distal to duplicon B (ZNF74) which identifies a normal 200kb NotI fragment and a rearranged 650kb fragment. A probe proximal to duplicon A detects the same rearrangement fragment, confirming that the DEPs are located within A and B. Isolation of the 650kb region of the gel from an A-B deletion and PCR analysis demonstrates co-migration of markers which flank the deletion proximal to A and distal to B, confirming that the novel band contains the DEPs. Similar studies for the A-C deletions with a probe distal to duplicon C and for the C-D deletion with a probe proximal to the C duplicon demonstrate rearrangement fragments by pulsed field gel electrophoresis analysis. These studies provide the first definitive evidence for two distinct duplicons and several recurring variant deletion endpoints within the 3 Mb 22q11.2 deletion region.

Program Nr: 387 from the 1999 ASHG Annual Meeting

Automated FISH spot counting by laser scanning cytometry detects homologous pairing of imprinted chromosomal domains. *J.M. LaSalle, C.P. Lagdamen.* Medical Microbiol & Immunology, Univ California Davis Sch Med, Davis, CA.

Interphase chromosome dynamics are important in the epigenetic regulation of gene expression. Homologous pairing of chromosome 15q11-13 regions occurs exclusively in the late S phase of the cell cycle and is deficient in cells from patients with the imprinting disorders Angelman (AS) and Prader-Willi's (PWS) syndromes. However, further investigations have been limited by the laborious method for detecting chromosome pairing by cell sorting and confocal microscopy. A novel technology for the automated detection and quantitation of fluorescent in situ hybridization (FISH) signals is the laser scanning cytometer (LSC), a microscope-based cytometer capable of rapid multiparameter analyses. We have developed a method to simultaneously determine cell cycle stage and FISH spot pairing by LSC. Normal human lymphocytes were hybridized with direct-labeled probes for either chromosome 15 or 12 and analyzed by LSC for FISH and DNA content. Due to its two-dimensional low resolution scanning, the LSC analysis undercounted the number of FISH spots by about 10%. Therefore, one-spot nuclei that contained one rather than two overlapping FISH signals were gated out of the analysis by using the area and fluorescent integral of the FISH signals from two-spot nuclei as a standard. Gates were set separately for each cell cycle stage, as the intensity of each signal doubled following replication. Using this approach, homologous pairing of D15Z1 was detected in more than 30% of the nuclei in late S phase, while other cell cycle stages showed less than 15% pairing. For the D12Z3 negative control, pairing was less than 15% in all cell cycle stages. These results are comparable to those achieved by the previous method but can be performed in approximately 10-fold less time. In future studies, the LSC-based detection of homologous pairing will be used to examine additional AS and PWS patients, to perform a genome-wide search for homologous pairing on other chromosomes, and to test homologous pairing in other tissues and species. This research should also have widespread applications in prenatal and cancer cytogenetics for automated multiparameter analyses on limited cell samples.

Program Nr: 388 from the 1999 ASHG Annual Meeting

Comparative genomic hybridization (CGH) in combination with flow cytometry improves the accuracy of cytogenetic analysis of spontaneous abortions (SAs). *B.L. Lomax[1], S.S. Tang[1], E. Separovic[1], T. Thomson[2], D.K. Kalousek[1]*. 1) Dept.of Path., Univ.of B.C., Canada; 2) Dept.of Lab.Med., B.C.Cancer Agency, Canada.

Chromosomal abnormalities account for approximately 60% of early pregnancy losses. Conventional cytogenetic analysis of SAs is dependent on tissue culture and subject to tissue culture failure and overgrowth of maternal cells. CGH represents an alternative cytogenetic technique capable of detecting chromosomal imbalances. CGH overcomes technical problems associated with tissue culture and in combination with flow cytometry can theoretically detect all numerical and unbalanced structural chromosomal abnormalities associated with SAs.

Routine cytogenetic and CGH analysis was performed on tissues samples from 175 SAs. All samples shown to be chromosomally balanced by CGH were analyzed by flow cytometry to determine ploidy. By conventional cytogenetic analysis, 56 (32%) demonstrated diploidy, 97 (55.4%) demonstrated aneuploidy and 22 (12.6%) failed. By CGH/flow analysis, 57 (32.6%) demonstrated diploidy, 111 (63.4%) demonstrated aneuploidy and 7 (4%) failed. Of the 149 cases successfully analyzed by both methods, there was a 93.2% correlation of the results. Discrepancies between the cytogenetic and CGH/flow results occurred in 10 cases. Six cases produced a 46,XX karyotype by cytogenetic analysis while CGH/flow demonstrated aneuploidy/polyploidy thereby indicating maternal contamination of the tissue cultures. In 2 cases, where tetraploidy was demonstrated by cytogenetics and diploidy by flow cytometry, tissue culture artifact is implied. In 2 unexplained discrepant cases, where the findings by CGH and cytogenetics differed, mislabeling of the samples is suspected and follow-up is in progress.

Our results demonstrate that CGH in combination with flow cytometry can readily identify aneuploidies associated with SAs, including trisomy, monosomy, gains and losses of partial chromosomes, triploidy and tetraploidy. CGH has a lower failure rate than conventional cytogenetic analysis and together with flow cytometry provides more accurate results by avoiding maternal contamination and tissue culture artifacts.

Program Nr: 389 from the 1999 ASHG Annual Meeting

Assessment of the relationship between size of deletion and level of developmental delay in cri-du-chat syndrome using a novel set of 5p FISH probes. *R.C. Marinescu[1], E.M. Dykens[2], R.M. Hodapp[3], D. Grady[4], X.-N. Chen[5], J. Overhauser[1]*. 1) Dept. of Biochem/Mol Pharm, Thomas Jefferson Univ, Philadelphia, PA; 2) Neuropsychiatric Inst, UCLA, Los Angeles, CA; 3) Graduate School of Education and Information Studies, UCLA, Los Angeles, CA; 4) Dept. of Biol Chem, UCI, Irvine, CA; 5) Med Genetics Birth Defects Center, Cedars-Sinai Medical Center, Los Angeles, CA.

Cri-du-chat syndrome is a segmental aneusomy syndrome that is associated with a deletion of the short arm of chromosome 5. A set of minimally overlapping YAC clones that span 5p as well as a 5p-telomeric BAC clone were developed to assess 110 cri-du-chat syndrome individuals with previously determined de novo terminal deletions of 5p. Of the 110 samples, 103 had terminal deletions, 4 had interstitial deletions, one had had an unbalanced translocation and no deletion could be detected in two samples. Developmental assessment was performed on 50 of these individuals with FISH-confirmed terminal deletions using the Vineland Adaptive Behavior Scales test. Composite Vineland Scores ranged from 20 - 75. In general, the communication score was higher than the composite score. Comparison of the size of the deletion with the composite Vineland score, as well as the Vineland Communication score, demonstrated that there was no correlation between the size of the deletion and the level of developmental delay. Many of the individuals scored at the basal level of 20. Of the individuals with scores higher than 50, most were between the ages of 1 and 10. Therefore, the age at the time of testing does appear to play a role in the level of delay that is observed.

Program Nr: 390 from the 1999 ASHG Annual Meeting

A Resource of Mapped BAC Clones for Cancer Chromosome Aberrations Identification. *N.J. Nowak[1], J.M. Conroy[1], G.P. Caldwell[1], J.J. Catanese[1], G. Schuler[2], B. Trask[3], D.R. Bentley[4], G. Shen[5], P.J. de Jong[1].* 1) Dept Cancer Genetics, Roswell Park Cancer Inst, Buffalo, NY; 2) NLM, NIH, Bethesda, MD; 3) University of Washington, Seattle, WA; 4) The Sanger Centre, Hinxton, Cambridge, UK; 5) DCB, NCI, Bethesda, MD.

Cancer is a heterogeneous disorder, encompassing more than 100 diseases, the progression of which is thought to result from an accumulation of genetic alterations at numerous loci controlling growth and proliferation. As part of NCI's initiative (RFA: CA-98-005) and joint effort with Caltech and the University of Washington, we are generating a high density, genome-wide resource of mapped BAC clones. These clones are selected from the RPCI-11 human BAC library for their application as tools for FISH analysis of chromosomal rearrangements in human cancer. The short-term goal would provide cytogeneticists with an evenly spaced set of clones, mapped at high resolution to analyze tumor rearrangements using FISH on metaphase chromosomes. The long-term goal would provide cytogeneticists with a genome wide clone array as a CGH target for high-resolution analyses of tumor DNA. A genome wide BAC clone array is being assembled through screening of the human BAC library, RPCI-11, with 4,000 markers mapped on the G3 and GB4 radiation hybrid panels. Markers (STS/EST) are judiciously chosen at a spacing of less than 1 Mb, and "overgos" are designed and arrayed into probe pools for hybridization based screening covering 6 fold redundancy of the library. Thus far, we have recovered BAC clones for over 1500 markers on chromosomes 1-3, 5, 6, 10, 11, 18-22 and X (http://bacpac.med.buffalo.edu/human/overview.html). We have recovered an average of 1 BAC/1-2 Mb for these chromosomes. One BAC clone per marker is characterized by in situ hybridization experiments to establish its usefulness as a FISH probe and to provide additional independent confirmation of the map location. Application of these resources will build a database of chromosome aberrations with diagnostic and prognostic potential providing clinicians with new tools for improving the current classification system of tumors.

Program Nr: 391 from the 1999 ASHG Annual Meeting

X chromosome inactivation studies in mosaic trisomies. *M.S. Penaherrera[1], I.J. Barrett[2], C.J. Brown[1], D.K. Kalousek[2], W.P. Robinson[1]*. 1) Dept. Medical Genetics; 2) Dept. of Pathology, Univ British Columbia, Vancouver, BC, Canada.

Skewed X-chromosome inactivation (XCI) is frequently found in fetal tissues of individuals with mosaic trisomy when the trisomy has arisen by a meiotic event and is predominantly confined to the placenta. Our model to explain both the elimination of the trisomy from most fetal tissues and the increased rate of skewing is that, the majority of the ~20 cells comprising the embryonic pool at the time of XCI are trisomic and that these trisomic cells are subsequently eliminated from fetal tissues by selection. Under this model, we would expect that the fewer diploid cells present at the time of XCI, the more likely extreme skewing is to occur. Furthermore, the greater the proportion of trisomic cells in the original embryonic pool the more difficult it would be to completely eliminate them from all fetal tissues. We therefore hypothesize that extreme skewing may be more often associated with a poor outcome, due to the presence of low level fetal mosaicism. In this study, XCI status was evaluated in 16 cases of prenatally detected meiotic trisomy mosaicism, involving chromosomes 2, 4, 7,16 or 22 and predominantly confined to the placenta. Using a PCR based methylation test at the androgen receptor locus, 10/16 cases showed extreme XCI skewing (>90% inactivation of one allele) in blood or other diploid fetal tissues, as compared to 6/111 in the control population ($p<0.001$). Of the 6 cases without extreme skewing, 4 had a normal outcome, while a low birth-weight (<5%ile) was the only abnormality in the remaining two cases. In contrast, of the 10 cases with extreme skewing, a normal outcome was found in only 2 cases. In the remainder, outcome ranged from severe IUGR to fetal malformations and intrauterine or neonatal death. It is possible that fetal mosaicism was present in those cases with a poor outcome even if amniotic fluid results were negative for the trisomy. Further investigations are required to determine the if XCI status adds useful information to the prediction of pregnancy outcome in prenatally detected mosaic trisomy.

Program Nr: 392 from the 1999 ASHG Annual Meeting

The first case of a microdeletion on chromosome 10p13 gives further evidence for a contiguous gene syndrome in the DiGeorge syndrome 2 region. *A.M. Rauch[1], P. Lichtner[2], M. Zenker[3], S. Schuffenhauer[2], M. Hofbeck[3], U. Trautmann[1]*. 1) Inst. for Human Genetics, FAU Erlangen, Germany; 2) Abt. Medizinische Genetik, LMU Muenchen, Germany; 3) Children's Hospital, FAU Erlangen, Germany.

DiGeorge syndrome (DGS) is a developmental field defect characterized by hypoplastic thymus and parathyroid, conotruncal heart defects and facial anomalies. About 90% of patients have a 3 Mbp microdeletion in 22q11.2, which in turn shows an immense clinical variability. Due to several patients with cytogenetical visible deletions of chromosome 10p, a second DGS locus was postulated. Recently Schuffenhauer et al performed deletion mapping in patients with deletion 10p and defined a critical DGS2 region in 10p13 of about 1cM containing D10S547 and D10S585. However, a microdeletion involving this DGS2 region was not yet quoted. We report here the first observation of a microdeletion 10p in a patient with growth and mental retardation and minor facial anomalies resembling published patients with larger deletions of 10p. Despite muscular hypotonia, shortness of stature, microcepahly and marked delay in developmental milestones he had no medical problems. Especially there was no abnormality of heart, kidneys, hearing, vision, T-cells, immunoglobulins, parathormone, x-ray of skull and EEG. There was no aberration detectable with GTG-banding at a 850 band level or with FISH for loci D22S75, cHKAD26 and D22S425 in 22q11.2. FISH with two Pacs containing WI-2389 and D10S585, respectively, demonstrated a de novo deletion of the DGS2 critical region in 10p13. As adjoining Pacs from a contig (P. Lichtner, unpublished) were not deleted, the size of this novel microdeletion is estimated to be about 400 kb. STRP analysis of loci D10S547 and D10S585 was not informative. Based on the clinical symptoms of this patient and those reported in other patients with larger deletions of 10p13 a tentative map of phenocritical segments can be constructed. Our findings indicate that a microdeletion of the recently proposed DGS2 critical region is not sufficient to produce the complete DGS phenotype. We therefore conclude that the DiGeorge syndrome 2 is a contiguous gene syndrome.

Program Nr: 393 from the 1999 ASHG Annual Meeting

A common neocentromeric region identified in two “acentric” chromosomes derived from 9p in unrelated individuals. *D.L. Satinover[1], G.H. Vance[2], D.L. Van Dyke[3], S. Schwartz[1]*. 1) CWRU and University Hospitals of Cleveland,OH; 2) Indiana Univ. School of Med., Indianapolis, IN; 3) Henry Ford Hospital, Detroit, MI.

Over the past few years, several cases of “neocentric” marker formation have been reported in accessory chromosomes without detectable levels of alpha-satellite sequences. Neocentromere activation has occurred in a variety of different markers originating from both autosomal and sex chromosomes. However, little is still known about the mechanism leading to the activation of a neocentromere. To attempt to answer the critical question whether neocentromere activation occurs at random or at preferential genomic sequences, we have further characterized two neocentromeres derived from the short arm of chromosome 9. Although these are mirror image duplications with different breakpoints (9p21.1 and 9p12), the neocentromeric region is not at the axis of symmetry and only a single neocentromeric region is formed. Our study was designed to develop a BAC contig across the neocentromeric region and utilize both FISH with the BACs as well as immunofluorescence with CENP-C to define our neocentromeric regions. We localized both neocentromeres to 9p23. Utilizing a series of BACs within 9p23, the neocentromeric region has been delineated between D9S269 and D92028 and BACs 246A3 and 420J5 respectively. The CENP signal is sandwiched between BAC 246A3 and 420J5. Radiation hybrid mapping reveals a distance of 1.35 Mb between these markers. To date, our BAC contig has added an additional 15 STS and 13 new BACs within this region of 9p23. Additional BAC clones and STSs are currently being localized to our map to further refine the neocentromeric sequences present in each marker.

Our current studies of these two markers indicate that: (1) The neocentromere region can be sized to smaller than 1.35 Mb by radiation hybrid mapping; (2) A complete BAC contig of this region is currently under construction; and most importantly (3) We have shown that neocentromeres found in two acentric chromosomes derived from 9p share a similar putative neocentromeric region in 9p23. These are very engaging findings suggesting that specific regions will preferentially gain neocentromeric activity.

Program Nr: 394 from the 1999 ASHG Annual Meeting

Deficient histone H4 acetylation in Roberts Syndrome. *R.A. Schultz[1,2], L.C. Probst[1], L.D. McDaniel[1,2].* 1) McDermott Ctr, Rm NB 10.120, Univ TX Southwestern Med Ctr, Dallas, TX; 2) Department of Pathology, Univ TX Southwestern Med Ctr, Dallas, TX.

Roberts syndrome (RBS) is a developmental disorder characterized clinically by tetraphocomelia (symmetrical limb reduction), cleft lip and palate, prenatal and postnatal growth retardation, mental retardation, craniofacial malformations, and cardiac and renal defects. RBS cells exhibit in vitro phenotypes that include aberrant splaying or repulsion of the heterochromatic regions of metaphase chromosomes (HR), faulty chromosome segregation, and modest sensitivity to a spectrum of chemical and physical agents, particularly those which damage DNA. Previous results from our lab and from others suggest that the HR phenotype is a consequence of altered chromatin structure, possibly related to a defect in histone acetylation/deacetylation. It is noteworthy that the developmental disorder Rubinstein-Taybi Syndrome also involves a defect in a protein associated with histone acetylation. We have been characterizing RBS cells to gain further insights into associations between chromatin structure and development and to identify the RBS genetic defect. Data will be presented demonstrating that RBS cells are hypersensitive to the histone deacetylase inhibitor tricostatin A (TSA), a result consistent with a potential defect in deacetylation. However, using antibodies against specific acetylated forms of histone H4, we have identified a defect in lys16 acetylation in RBS cells as detected by reduced intensity of immunofluorescence staining. Quantitative evaluation of the results indicated that this was only a partial defect, as some histone H4(Ac16) acetylation was detected in RBS cells. The defect was documented in all three RBS cell lines examined, was specific for H4(Ac16), and could be observed at all stages of the cell cycle. The results suggest a relationship between TSA hypersensitivity and defective histone H4 acetylation.

Program Nr: 395 from the 1999 ASHG Annual Meeting

Late replication and histone deacetylation spreading in a human X;1 translocation. *S.S. Thomas[1], L. Hudgins[2], E.A. Keitges[3], C.M. Disteche[1]*. 1) Dept. of Pathology, Univ. of Washinton, Seattle, WA; 2) Div. of Medical Genetics, Univ. of Washington and Children's Hospital, Seattle, WA; 3) Dynacare, Seattle, WA.

An unbalanced translocation between the X chromosome and chromosome 1 was found in a child with multiple congenital abnormalities, including ventricular septal defects, hydrocephalus, seizures, growth retardation of prenatal onset and significant development delay. Breakpoints of the translocation were located at bands Xq21 and 1q21. By FISH analysis the XIST (X-inactive specific transcript) gene was found to be present on the derivative X close to the breakpoint. Late replication studies using BrdU-labeling late in S phase and Giemsa or BrdU antibody stainings revealed extensive but incomplete spreading of late replication. This is consistent with inactivation of the derivative chromosome including a large portion of the attached chromosome 1, which would explain survival of this child, who otherwise would be trisomic for most of the long arm of chromosome 1. Lack of acetylated histone H4 was previously shown to characterize the inactive X chromosome but there was no evidence of its spreading in human X/autosome translocations. Antibody to acetylayed histone H4 was applied to chromosome preparations from a lymphoblastoid cell line from the patient. Lack of staining was most prominent in the X portion of the derivative chromosome but was also observed in the chromosome 1 portion, in a pattern corresponding to the late replication pattern. The concordance between histone deacetylation and late replication in the derivative chromosome indicates that histone deacetylation can spread into autosomal material attached to the inactive X chromosome in a human X/autosome translocation..

Program Nr: 396 from the 1999 ASHG Annual Meeting

Spectral karyotyping (SKY) in combination with locus-specific FISH, a technique to simultaneously define genes and chromosomes involved in chromosomal translocations. *G. Tonon, A. Roschke, W.M. Kuehl, I.R. Kirsch.* Dept Genetics, Medicine Branch, National Cancer Institute, Bethesda, MD.

Genes critical for the origin and development of cancer have often been found proximate to chromosomal breakpoints. Identifying the genes that flank chromosomal reconfigurations is thus essential to the study of cancer cytogenetics. To simplify and expedite this identification, we have developed an approach, based on simultaneous spectral karyotyping (SKY) and fluorescence in situ hybridization (FISH) analyses. In SKY, each chromosome is defined by a single color, and rearranged chromosomes present two or more colors, corresponding to the different chromosomes participating in the rearrangement. The signal specific for the queried genes (FISH probe) was easily detectable in the metaphase spread, together with the signals from the painted chromosomes (SKY probes). This technique is rapid and simple. The concentration and size of the FISH probes for successful analysis covered a wide range. We have been able to identify the FISH signal with a 12.5 Kb probe at 10 mg/ml concentration, using only 0.1 mg of total probe. Two FISH probes can be used, therefore more than one gene can be tested in the same metaphase for its involvement in breakpoints. As a test of this method the myeloma cell line Karpas 620 was studied. This cell line has a complex karyotype, characterized by numerous chromosomal rearrangements. Using as a probe the constant region of the immunoglobulin heavy chain, we found six copies of this gene, all of them located coincident with translocations. Surprisingly, two of these copies were not located in chromosome 14 derivatives, where this gene normally resides, but at the breakpoint between chromosome 8 and 11, demonstrating an insertion of this gene and therefore a previously undetected chromosomal aberration. In summary, we demonstrate a technique that in a single step can localize specific genes and define their involvement in chromosomal breakpoints. This approach will be particularly useful for the analysis of complex karyotypes and for testing hypotheses arising from the flow of information derived from cancer gene expression studies.

Program Nr: 397 from the 1999 ASHG Annual Meeting

Clinical Outcome of Common Trisomies 13, 18, 20, and 21 Diagnosed through Amniocytes: A Collaborative Karyotype-Genotype Study. *R. Wallerstein[1,3], M-T. Yu[2,3], L.Y.F. Hsu[1,2,3], 23 North American Cytogenetic Laboratories[3]*. 1) Human Genetics Program, New York University, New York, NY; 2) Prenatal Diagnosis Laboratory of New York, New York, NY; 3) Collaborative Amniocentesis Mosaicism Project.

Karyotype-phenotype correlations of common trisomy mosaicism prenatally diagnosed via amniocentesis was reviewed in 305 new cases from a collaboration of North American cytogenetic laboratories. Abnormal outcome was noted in 10/25 (40%) cases of 47,+13/46, 17/31 (54%) cases of 47,+18/46, 10/152 (6.5%) cases of 47,+20/46, and in 49/97 (50%) cases of 47,+21/46. Risk of abnormal outcome in pregnancies with less than 50% trisomic cells and greater than 50% trisomic cells were: 26% (4/15) vs. 60% (6/10) for 47,+13/46, 52% (11/21) vs. 75% (6/8) for 47,+18/46, 4.5% (6/132) vs. 20% (4/20) 47,+20/46, and 45% (27/60) vs. 59% (22/37) for 47,+21/46. Phenotypically normal liveborns were observed with mean trisomic cell lines of 9.3% for 47,+13/46, 8.6% for 47,+18/46, 27% for 47,+20/46, and 17% for 47,+21/46. Concordance of confirmation study with original amniocentesis was observed in 6 out of 13 (46%) studies performed on pregnancies with 47,+13/46 mosaicism, 8/12 (66%) of 47,+18/46, 10/97 (10%) of 47,+20/46, and 24/54 (44%) of 47,+21/46. There were higher confirmation rates in pregnancies with abnormal vs. normal outcome: 47,+13/46 (50% vs. 44%), 47,+18/46 (100% vs. 33%), 47,+20/46 (66% vs. 7%), and 47,+21/46 (55% vs. 40%). Repeat amniocentesis is not helpful in differentiating clinical outcome due to very similar confirmation rates in both normal and abnormal cases. Fetal blood sampling may have a role in mosaic trisomy 13, 18, and 21 as the risk for abnormal outcome increases with positive confirmation: 1/5 (20%) normal cases vs. 5/8 (62%) abnormal cases. High resolution ultrasound examination(s) is recommended for clinical correlation and to facilitate genetic counseling.

Program Nr: 398 from the 1999 ASHG Annual Meeting

Short stature and premature ovarian failure loci in proximal Xp. *A.R. Zinn[1], R.L. Prueitt[1], P.R. Papenhausen[2], V.L. Roberts[3], J.L. Ross[4].* 1) McDermott Center & Dept. of Internal Medicine, UT Southwestern Medical School, Dallas, TX; 2) LabCorp of America, Research Triangle Park, NC; 3) Endocrine Associates of Florida, Longwood, FL; 4) Dept. of Pediatrics, Thomas Jefferson University, Philadelphia, PA.

Turner syndrome, the phenotype of human monosomy X, is thought to be due in large part to haploinsufficiency of genes that escape X-inactivation. Recently we identified a critical region at Xp11.2-p22.1 for short stature, premature ovarian failure (POF), high arched palate, and autoimmune thyroid disease by genotype/phenotype correlation of 28 girls and women with partial deletions of Xp (Zinn et al. AJHG 1998 63:1757). We now report a 31 year old woman with short stature, secondary amenorrhea, and a *de novo* interstitial deletion of Xp11 partly overlapping the previously defined critical region. The patient's height was 143.5 cm, 3.3 SD below the population mean and 1.7 SD below her midparental target height. Molecular cytogenetic studies of peripheral blood leukocytes showed no evidence of mosaicism and confirmed that the deletion is interstitial, with proximal breakpoint between *ZXDB* (Xp11.21) and *DXS423E* (Xp11.22) and distal breakpoint between *SYP* (Xp11.23) and *SYN1* (Xp11.3). The deletion spans 5-10 Mb and is to our knowledge one of the smallest interstitial deletions associated with characteristic Turner syndrome features. Both copies of the Xp pseudoautosomal short stature gene *SHOX* are present, supporting our previous suggestion that haploinsufficiency of the pseudoautosomal *SHOX* gene does not account for the entire growth deficit in Turner syndrome. The deletion in our patient extends proximal to a previously reported interstitial deletion (Xp11.23-p22.11) in a mother and two daughters with isolated short stature reported by Herva et al. (AJMG 1979 3:43). Our data are consistent with these authors' hypothesis that there are distinct stature and POF loci situated in proximal Xp. Additional studies will refine the interval most likely to contain the responsible gene(s).

Program Nr: 399 from the 1999 ASHG Annual Meeting

The Necker Human Embryos Collection. *T. Attié-Bitach[1], F. Encha-Razavi[1], I. Kirillova[2], A-L. Delezoide[1], C. Esculpavit[1], D. Esnault[1], M. Vekemans[1]*. 1) Dept de Génétique & INSERM U-393, Hopital Necker, Paris, FRANCE; 2) Institute for Hereditary Diseases, Minsk, BELARUS.

After many years of descriptive embryology, our knowledge of human development remains very limited because human embryo research is restricted by both practical and ethical considerations. The mouse is a good model that can teach us an enormous amount about human development. But there are now good arguments to suggest that future developmental research should include also studies in human embryos. Collection of human embryos was obtained from 546 RU486 induced abortions between January 1996 and December 1998. The embryos have been collected according to the French law and the French Ethical Committee recommendations. Whole gestational sacs were placed either in 4% paraformeldehyde (PFA) or saline solution for transport to the laboratory for stereomicroscope assessment of Carnegie stage and dissection where required. Photography and image capture records were made at the same time. Three hundred and nineteen embryos (60%) were obtained of which 6 were lost during inclusion, 38 were damaged, 11 judged abnormal, 41 were frozen, 64 were used for electronic microscopy studies and 160 were paraffin embedded. The embryos spanned Carnegie stages 9-21 which correspond to a wide window of human organogenesis A potential concern is the uncertainty as to when embryonic demise actually occurs as autolysis may reduce mRNA retention, producing suboptimal expression studies. Our experience is that mRNA is retained and in situ hybridization experiments for several genes (RET, GDNF, SOX10, PAX2, SHH, JAGGED1...) are successful. Chromosome analysis of 37 embryos was obtained using FISH analysis with chromosomes 16, X and Y probes. No abnormal result has been noted to date. In conclusion, we believe that there is a need for future research on human embryos. The collection of intact embryos from medical termination is possible. It provides embryos spanning a wide window of human development. Our experience shows that molecular and tissue in situ hybridization studies are possible on this material. We are making material from our collection available for collaborative gene expression studies.

Program Nr: 400 from the 1999 ASHG Annual Meeting

Physical mapping of the MKS2 locus in Meckel syndrome. *L. Benedetti-Clech[1], J. Roume[2], M. Le Merrer[1], V. Cormier-Daire[1], J. Augé[1], A. Munnich[1], F. Encha-Razavi[1], N. Dagonneau[1], M. Vekemans[1], T. Attié-Bitach[1].* 1) Dept de Génétique & INSERM U-393, Hopital Necker, Paris, FRANCE; 2) Service de Foeotopathologie, Hopital St Antoine, Paris, FRANCE.

Meckel syndrome (MKS) is a rare, lethal autosomal recessive disorder characterized by occipital meningoencephalocele, multicystic dysplasia of the kidney, fibrotic changes of the liver and postaxial polydactyly. MKS1 has been mapped to chromosome 17q21-q24 in Finnish pedigrees. However, genetic heterogeneity at this locus has been shown in several Western European and North African families. More recently, based on homozygosity mapping in seven inbred families from North Africa and the Middle East, a second locus, MKS2, has been mapped to chromosome 11q13 between markers D11S916 and D11S937. Interestingly, in this form of the disease, fetuses show a distinctive CNS malformation pattern, including a prosencephalic dysgenesis, an occipital exencephalocele and a rhombic roof dysgenesis with absent tectum of the brain and agenesis/ dysgenesis of the cerebellar vermix. In order to refine the mapping of the MKS2 locus, we constructed a physical map using 14 overlapping yeast artificial chromosome (YAC) clones. Microsatellite markers of the YAC contig were subsequently studied in the families. This allowed us to restrict the homozygous region to a 0 cM genetic interval between markers D11S4079 and D11S937. The critical region is included in two overlapping YACs (1 Mb). We are currently constructing a bacterial artificial chromosome (BAC) contig by screening the RPCI-11 BAC library with the markers closely linked to the MKS2 locus. In addition, using a complementary strategy to identify candidate genes, four expressed sequence tags were excluded (including the ARIX gene). Another candidate gene located on the contig, possibly in the homozygous region, is being sequenced in MKS2 patients. In conclusion, at least two mutant genes are associated with the MKS phenotype. The identification of these genes should contribute importantly to our understanding of brain, kidney and limb human embryonic development.

Program Nr: 401 from the 1999 ASHG Annual Meeting

Prenatal diagnosis and carrier detection of albinism. *A. Blumenfeld[1], I. Bejarano-Achache[1], A. Rosenmann[2].* 1) Molecular Biol Unit, Hadassah Univ Hosp; 2) Michaelson Inst for Prevention of Blindness, Hadassah Med Org, Jerusalem, Israel.

Oculocutaneus Albinism Type I (OCA I) is an autosomal recessive disease that comprises several phenotypes of hypopigmentation and low vision up to legal blindness. OCA I is caused by lack of tyrosinase activity, and in most cases mutations were found in the tyrosinase gene (TYR). Due to the severe visual handicap, affected families seek genetic counseling and prenatal diagnosis.

Prenatal diagnosis of OCA I was performed at Hadassah since 1989 by light and electron microscopic examination of melanogenesis in fetal scalp biopsies. Thirty one prenatal tests were performed, and five affected fetuses were diagnosed. Since 1997 we offer molecular genetic tests for carrier detection, and prenatal diagnosis of OCA I. Five molecular prenatal tests were performed, and two affected fetuses were diagnosed. In three families mutations in the two copies of the TYR gene were detected in the propositus; one is a compound heterozygote, and two were homozygote. Each of the normally pigmented parents was found to carry one mutation, and the fetuses were tested by direct screening for the mutation(s). In the two other families, only one mutation was detected in the propositus. Three polymorphisms within the TYR gene were used to determine haplotypes of the albino child and both parents. The prenatal test was based on a combination of mutation detection and haplotype analysis.

We have screened 115 unrelated Israeli albinos and their families with twelve mutations previously described in our population, or in non-Israeli Caucasians. In certain ethnic groups we found at least one mutation in all, and two mutations in over 90 percent of albinos. In these groups we offer mutation detection to normally pigmented unrelated spouses of albinos and of diagnosed carriers pertaining to OCA I families.

Based on our findings, prenatal and carrier molecular diagnosis of OCA I becomes possible in an increasing number of Israeli albino families.

Program Nr: 402 from the 1999 ASHG Annual Meeting

Chromosome 21 deletion and cortical dysgenesis: Intersectin (ITSN) is expressed in subsets of post-mitotic neurons in the developing brain. *X-N. Chen[1], D.P. Huynh[2], Y. Huo[1], Z-Y. Shi[1], M. Hattori[3], Y. Sakaki[3], S.M. Pulst[2], J.R. Korenberg[1]*. 1) Med Genet, Cedars-Sinai Med Ctr, UCLA, CA; 2) Dept Medicine, Cedars-Sinai Med Ctr, UCLA, CA; 3) Human Genome Research Group, RIKEN GSC, Japan.

To study the mechanisms responsible for Down Syndrome (DS) and aneuploidy of chromosome 21, we have characterized genes in the candidate region for cortical dysgenesis (CD) and deletion 21. The gene encoding intersectin (ITSN) spans more than 300 kb of the 5 Mb candidate region, includes transcripts specific to the brain, and domains (EH, SH3, GEF, PH), and is involved in clathrin-mediated endocytosis. As first step, we studied its expression and deletion in 16 individuals using FISH. In the fetal and adult human, we used multiple-tissue Northern blots with 20 exon and domain-specific cDNA fragments and in the developing and adult mouse, domain-specific immunocytochemistry. For human expression, novel patterns of fetal- and brain-specific transcripts were found. These include a 4.5 kb moiety specific to the fetus, that includes all domains of ITSNs with high expression in liver and possibly significant for hematopoesis. A 2.0 kb form is also largely specific to fetus and appears to begin with the 5UTR encoded by exon 1 but excludes the EH domains and includes alternatively spliced forms of the SH3 domains. Exon1 is particularly highly expressed in fetal brain and alpha helix in fetal liver, kidney, brain and lung, suggesting further alternative splicing for isoforms involved in early development. For mouse, antibodies to SH3 (Src homology 3) domains were generated and used to study the brain in the developing and adult mouse. ITSN immunoreactivity was found beginning on E11 in subsets of post-mitotic neurons of the cortex, in axonal bundles and in the myocardium; on E14-16, in cellular subsets of the dermis. In the adult mouse brain, staining was also found in a subsets of neurons in the cortex, hippocampus, thalamus and the axonal bundles but not in cerebellum or in dendritic processes. These findings suggest a role for ITSN in the early development of the brain, a possible contribution to CD in deletion for 21, to the mental retardation found in DS.

Program Nr: 403 from the 1999 ASHG Annual Meeting

Mapping gene expression domains and neuronal cell differentiation during human embryonic forebrain development. *D.M. Hagan[1], S. Lisgo[1], T. Strachan[1], D. Davidson[2], R. Baldock[2], M. Stark[2], E. Boncinelli[5], M. Clement-Jones[1], D. Wilson[1], S.C. Robson[3], G. Clowry[4], S. Lindsay[1]*. 1) Human Genetics, Univ. of Newcastle Upon Tyne, Newcastle, UK; 2) MRC Human Genetics Unit,Edinburgh,UK; 3) Dept. of Fetal Medicine. Univ. of Newcastle upon Tyne,UK; 4) Dept. of Child Health, Univ. of Newcastle upon Tyne, UK; 5) Lab. of Molecular Biology of Development, Milan, Italy.

The human forebrain is crucially important to higher order functions and is affected by many significant and disabling genetic or congenital disorders. From a very early stage of development the human forebrain is unmistakably different from that of other mammalian species suggesting that we need to study forebrain development directly in human. Fundamental to normal brain development is the differentiation of progenitor cells along either neuronal or glial pathways, processes which are under genetic control. Relatively little however is known about the molecular mechanisms controlling development and differentiation of the embryonic forebrain.

We examined the expression of a range of genes in embryonic forebrain at human CS18 (44 post-ovulatory days). The genes investigated are either implicated in mammalian forebrain development and/or are causative genes for disorders with abnormal brain pathology and include *PAX6, PAX3, SHH,EMX1,EMX2,OTX1* and *OTX2*. We have also investigated neuronal and glial cell differentiation immunohistochemically. All of the genes investigated were abundantly expressed at CS18. Specific domains of expression were identified within the developing forebrain. Expression boundaries did not necessarily conform to any one underlying morphological feature concurring with the concept of gene expressing subregions or compartments within the developing forebrain neuroepithelium. To aid visualisation and interpretation of the gene expression and immunohistochemical patterns, computer-based 3 dimensional reconstructions of the data are being prepared. This study should provide a better understanding of the complex organisation and gene interactions of the developing human forebrain.

Program Nr: 404 from the 1999 ASHG Annual Meeting

Improved Growth and Cultured Neuron Viability In Partial Interferon-Alpha/Beta and -Gamma Receptor Knockout Trisomy 16 Mouse Fetuses. *D.M. Hallam, T.N. Heffernan, L.E. Maroun.* Department Of Medical Microbiology And Immunology, Southern Illinois University School Of Medicine, Springfield, IL.

The trisomy 16 mouse fetus is a well studied model for Down syndrome (Trisomy 21), the leading genetic cause of mental retardation in the newborn population. Human chromosome 21 and mouse chromosome 16 each carry a large cluster of genes that code for components of the interferon (IFN) -alpha/beta and -gamma receptors, and Down syndrome cells display significantly increased sensitivity to IFN action. We have previously reported that in utero anti-IFN IgG treatment of mice pregnant with trisomy 16 fetuses results in a significant improvement in trisomy 16 fetus growth and morphology [Teratology 51 (1995) 329-335] and that anti-IFN-gamma immunoglobin treatment can prevent the premature death of trisomy 16 fetal mouse cortical neurons in culture [Neurosc. Lett. 252 (1998) 17-20]. As a measure of growth, trisomy fetus crown-to-rump length was measured relative to euploid littermates. To evaluate neuron viability, cortical neurons were cultured in serum-free media for six days and quantitated by photographic analysis. Apoptosis was assessed using the annexin-V apoptosis assay and analyzed by flow cytometry. We have now used interferon receptor subunit knockout mice to produce mouse fetuses that carry three #16 chromosomes and one copy each of disabled IFN-gamma receptor and IFN-alpha/beta receptor component genes. We observed that this partial interferon receptor knockout trisomy mouse fetus has significantly improved growth, and yields cortical neurons whose viability and frequency of apoptosis is the equivalent of that seen in their euploid counterparts.

Program Nr: 405 from the 1999 ASHG Annual Meeting

The Prenatal Diagnosis of the Skeletal Dysplasias. *D. Krakow[1], M. Priore[1], L. Rimoin[1], D. Carlson[1], R. Lachman[2], D. Rimoin[1]*. 1) Division of Genetics, Cedars-Sinai Medical Ctr, Los Angeles, CA; 2) Department of Radiology, Harbor-UCLA Medical Ctr, Torrance, CA.

The osteochondrodysplasias, including the dysostoses, are a genetically heterogeneous group of disorders characterized by abnormalities in the patterning, development, growth and maintenance of cartilage and bone. While it is difficult to determine the precise incidence of these disorders, the overall birth incidence for this group of conditions approaches 1/10,000. With the increasing use of ultrasound many prenatal-onset skeletal dysplasias are now being recognized, but a precise diagnosis determined by ultrasound findings can be challenging. Through the International Skeletal Dysplasia Registry (ISDR) we undertook two studies. First we retrospectively analyzed over 750 cases which were ascertained in the prenatal period by either ultrasound or radiographs. In 70 % of the cases the referring diagnosis was incorrect, and the diagnosis correct in approximately 30%. The most common final diagnoses made by the ISDR were osteogenesis imperfecta type II (20%), thanatophoric dysplasia (11%), and achondrogenesis II (8.2%). When compared to the referring diagnosis, these three relatively common diagnoses were made correctly less than half the time. We have prospectively ascertained ultrasounds in over 80 cases referred to us for a precise diagnosis. We have determined lethal versus non-lethal in 100% of the cases, and overall, we have generated the correct diagnosis based on ultrasound findings in almost 80% of cases. The criteria that we employ for diagnosis are based on those used for radiographic diagnosis. For example, these include the size and shape of the long bones, the location of the bony abnormality, mineralization and associated congenital anomalies. Our ultrasound referred cases are then analyzed by radiographs, histomorphology and molecular diagnosis when applicable. This is of critical importance since the most accurate diagnosis must be made for the further counseling of the families. We believe that with careful review and an understanding of the radiographic findings in these disorders, an accurate diagnosis can be obtained in the prenatal period.

Program Nr: 406 from the 1999 ASHG Annual Meeting

Mammalian orthologs of invertebrate sex differentiation genes are androgen-responsive in motor neurons. *A.P. Lieberman[1], D.L. Friedlich[2], K.H. Fischbeck[1]*. 1) Neurogenetics Branch, NINDS, NIH, Bethesda, MD; 2) HHMI-NIH Research Scholars Program.

The androgen receptor is a ligand-activated transcription factor that is expressed in motor neurons of the brainstem and spinal cord. Activation of the androgen receptor induces trophic effects in motor neurons in vivo. We sought to identify androgen-responsive genes in motor neurons in hopes of identifying novel pathways activated by androgens in these cells. Mouse motor neuron-neuroblastoma hybrid cells permanently transfected with the full-length normal human androgen receptor respond to stimulation with a synthetic androgen (R1881) by showing increased survival and enlarged soma size. This in vitro model thus recapitulates the trophic actions of androgens on motor neurons. Using this model and a PCR-based subtractive hybridization approach, we identified ten genes that are expressed at higher levels following androgen stimulation. Two of these genes, tra-2 alpha and fox-1, encode RNA-binding proteins that are mammalian orthologs of invertebrate sex differentiation pathway components. Tra-2 (transformer-2) is an enhancer of RNA splicing involved in Drosophila and C. elegans sex differentiation, while fox-1 (feminizing on X-1) functions upstream of tra-2 in C. elegans. Expression of both genes is increased in our model by stimulation with R1881 in a time and dose-dependent fashion. We isolated full-length cDNAs for mouse tra-2 alpha and fox-1, and found that they are widely expressed in mouse tissues. Tra-2 alpha mRNA is subject to complex, tissue-specific alternative splicing. The full-length cDNA, consisting of two RS domains and the RNA recognition motif, is the dominant form expressed in brain and is the major androgen-responsive transcript in our in vitro model. Mouse fox-1 protein is 90% identical to the human fox-1 protein, and is 75% identical to C. elegans fox-1 over an 84 amino acid stretch that encodes the RNA binding domain. Our data suggest that a pathway consisting of mammalian orthologs of invertebrate sex differentiation genes is androgen-responsive in motor neurons.

Program Nr: 407 from the 1999 ASHG Annual Meeting

Expression of the short stature homeobox gene, *SHOX*, correlates with skeletal abnormalities in Turner syndrome. *S. Lindsay[1], M. Clement-Jones[1], E. Rao[2], R.J. Blaschke[2], A. Zuniga[3], R. Zeller[3], S.C. Robson[4], T. Strachan[1], G.A. Rappold[2]*. 1) School of Biochem. & Genetics, Univ Newcastle upon Tyne, Newcastle on Tyne, England; 2) Institute of Human Genetics, Ruprecht-Karls-University, Heidelberg, Germany; 3) Dept. of Developmental Biology, Univ Utrecht, Utrecht, The Netherlands; 4) Dept. of Fetal Medicine, Univ Newcastle upon Tyne, Newcastle upon Tyne, England.

Turner syndrome is characterized by short stature and is frequently associated with a variable spectrum of somatic features: gonadal dysgenesis, heart and renal abnormalities and a range of skeletal anomalies which include Madelung deformity, a key feature of Leri-Weill syndrome. Defects of the pseudoautosomal homeobox gene SHOX were previously shown to lead to short stature and Leri-Weill syndrome (1,2), and haploinsufficiency of SHOX was suggested to cause the short stature phenotype in Turner syndrome (3,4). Despite exhaustive searches, no direct murine orthologue of SHOX is evident. SHOX is however closely related to the SHOX2 homeobox gene on 3q which has a murine counterpart, Og12x (5,6). We analysed SHOX and SHOX2 expression during human embryonic development, and referenced the expression patterns against those of Og12x. The SHOX expression pattern in the limbs and first and second pharyngeal arches not only explain SHOX related short stature phenotypes but also for the first time provide strong evidence for the causal involvement of this gene in the development of additional Turner stigmata. 1. Belin, V. et al. Nature Genet. 19, 67-69 (1998). 2. Shears, D.J. et al. Nature Genet. 19, 70-73 (1998). 3. Rao, E. et al. Nature Genet. 16, 54-62 (1997). 4. Ellison, JW et al. Hum. Mol. Genet. 6, 1341-1347 (1997). 5. Blaschke, R.J. et al. Proc. Nat. Acad. Sci. 95, 2406-11 (1998). 6. Semina, E.V. et al. Hum. Mol. Genet. 7, 215-22 (1998).

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Program Nr: 408 from the 1999 ASHG Annual Meeting

Gene Expression Profiles of Developing Forelimbs and Hindlimbs: Investigating the Genetic Pathways of Limb Identity. *E.H. Margulies*[1], *J.W. Innis*[1,2]. 1) Departments of Human Genetics and; 2) Pediatrics, University of Michigan, Ann Arbor, MI.

Dramatic morphological differences exist between the upper and lower limbs of animals. While most of the genes known to be involved in limb development are similarly expressed in both structures and conserved between animal species, some genes, such as the transcription factors *Pitx1*, *Tbx5*, and *Tbx4*, are differentially expressed and have recently been identified as important components of the genetic pathways determining limb identity. However, upstream regulators and downstream targets of these transcription factors are predominantly unknown. A comprehensive gene expression profile from the developing forelimb and hindlimb would 1) provide a novel resource for determining the genetic hierarchies regulating limb development and identity 2) allow for an evolutionary comparison of limb patterning between other animal models 3) identify candidate genes for the nearly four dozen inherited human limb malformation syndromes that affect either the upper or lower limbs and 4) identify the subset of all genes specifically expressed in the developing vertebrate limb.

To identify differentially expressed genes between developing forelimbs and hindlimbs, Serial Analysis of Gene Expression (SAGE) was used to generate a comprehensive, unbiased and quantitative gene expression profile from populations of mRNA purified from mouse E11.5 forelimbs and hindlimbs. 11,776 forelimb and 11,806 hindlimb SAGE Tags representing 10,793 different transcripts were sequenced. Using the test statistic developed by Audic and Claverie (1997), 69 transcripts, including *Pitx1*, were identified as differentially expressed ($p < 0.05$) and 30 have a greater than 5-fold difference. Whole-mount *in situ* hybridizations are being used to confirm the gene expression differences detected by SAGE and to identify other regions of expression in the embryo. This is the first comprehensive analysis of gene expression in the developing vertebrate limb and will provide a valuable framework for investigating the genetic interactions involved in limb identity and development.

Program Nr: 409 from the 1999 ASHG Annual Meeting

Novel function of *LMX1B*during kidney and limb development and pathogenesis of nail patella syndrome. *R. Morello[1,2], S.D. Dreyer[1], K. Oberg[1,3], B. Lee[1]*. 1) Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Advanced Biotech. Center, Genoa, Italy; 3) Pathology, Loma Linda Univ., Loma Linda, CA.

Nail patella syndrome (NPS) is a dominantly inherited skeletal malformation syndrome characterized by nail, skeletal and renal abnormalities. Mutations in a LIM homeodomain protein (LIM HD) specifying dorsal limb mesenchymal cell fate, *LMX1B*, result in NPS. Interestingly, *Lmx1b-/-*mice duplicate the human condition. Here, we performed transactivation studies to determine whether a dominant negative effect might underlie the NPS phenotype in human vs. mice. In addition, we identify potential downstream targets of *Lmx1b*in joint and kidney development. Transfection of mutant *LMX1B*constructs containing homeodomain, LIM domain, and activation domain mutations each exhibited dose-related loss of transactivation of target reporters without dominant negative effect on wild type *LMX1B*action in mixing experiments. This was unaffected by cotransfection of LIM HD interacting proteins, E47 and Ldb1. We tested markers of joint development *Gdf-5*and *sFRP2*as candidate targets of *LMX1B*action. While *Gdf-5*expression is normal in the mutant mice, *sFRP2*expression appears to be symmetrically distributed in the dorsal-ventral axis of the *Lmx1b-/-*limb. This effect is likely due to the ventralized pattern of the dorsal structures in mutant limbs. In contrast to *LMX1B*function in limb development, its role in kidney development was discovered by correlation with the renal phenotype in NPS. Based on histological abnormalities in glomerular basement membranes (GBM) of NPS patients, we hypothesized that *LMX1B*might play a role in GBM development. We show that *Lmx1b*localizes to the glomeruli from E15.5 and newborn mice. Expression of *sFRP2*, also a marker of glomerular epithelium, and of a1(IV) collagen were intact. However, mutant kidneys showed marked absence of a3(IV) and a4(IV) collagen expression consistent with histologic GBM abnormalities and the clinical albuminuria observed in NPS patients. Moreover, this suggests a novel and integral role for*LMX1B*in the coordinated transcriptional switching of a1(IV) and a2(IV) to a3(IV) and a4(IV) expression during GBM morphogenesis.

Program Nr: 410 from the 1999 ASHG Annual Meeting

Detection of male and female fetal DNA in maternal plasma by multiplex fluorescent PCR amplification of short tandem repeats (STRs). *B. Pertl[1], O. Samura[2], A. Sekizawa[2], D.W. Bianchi[2]*. 1) Dept of Ob/Gyn, Univ of Graz, Graz, Austria; 2) Division of Genetics, Dept of Pediatrics,Tufts Univ Sch of Med, Boston, MA.

Background: Bi-directional cell trafficking of fetal and maternal cells occurs during pregnancy. Almost all-prior investigations have focused on complete and intact fetal cells in the maternal circulation. Recently, reports by Lo et al. have suggested that large amounts of cell-free fetal DNA are detectable in maternal serum and plasma. However, prior reports only detected Y chromosomal sequences in male fetuses. The goal of this study was to develop a fluorescent PCR assay for the detection of both male and female fetal circulating DNA in the plasma of pregnant women.

Methods: Maternal DNA was extracted from plasma samples of 12 pregnant women at term and newborn umbilical cord blood DNA was extracted to confirm fetal polymorphisms. The mother/child pairs were genotyped at 9 different polymorphic short-tandem repeat (STR) loci. Multiplex fluorescent PCR was used to detect fetus-specific alleles in the corresponding maternal plasma samples.

Results: Fetus-specific alleles were found in all maternal plasma samples studied. Using these polymorphic STRs every mother-child pair was informative in at least 4 out of 9 loci. Paternally inherited fetal alleles were detected in 80% of informative STRs.

Conclusions: Compared to other fetal DNA detection systems that use fetus-derived Y sequences to detect only male fetal DNA in maternal plasma, our technique can be applied to both male and female fetuses. This may expand the clinical applications of the use of fetal DNA in maternal plasma.

Program Nr: 411 from the 1999 ASHG Annual Meeting

Preimplantation diagnosis for translocations by karyotyping single blastomeres and second polar body. *Y. Verlinsky, S. Evsikov, J. Cieslak, V. Ivakhnenko, G. Wolf, C. Strom, A. Kuliev.* Reproductive Genetics Inst, Chicago, IL.

Preimplantation genetic diagnosis (PGD) for translocations is currently based on interphase FISH analysis of blastomeres or the first polar bodies, containing metaphase chromosomes, which, however, still have limitations for testing of some translocations and for complete karyotyping. To improve the accuracy of PGD for maternal translocations by the first polar body, sequential karyotyping of the second polar body or single biopsied blastomeres is required. Visualization of the chromosomes of single blastomeres is also needed for accurate PGD for paternally derived translocations. To obtain metaphase chromosomes from the human second polar body, we injected the polar body nucleus into the cytoplasm of an enucleated oocyte, which was followed by treatment with okadaic acid to induce premature chromosome condensation. Analyzable chromosomes were obtained in 66% of cases, and the method was applied for PGD of the following maternal translocations: t(17;18)(q32;q23); t(12;18)(p13.31;q21.32). To visualize chromosomes of single human blastomeres, we fused biopsied blastoneres with enucleated or intact mouse zygotes and fixed the resulting heterokaryons at the metaphase of the first cleavage division, or treated with okadaic acid to induce premature chromosome condensation. The method was 91% efficient and has been applied for PGD of the following paternally derived reciprocal translocations: t(6;7)(q23;q36); t(11;22)(q23.3;q11.2); t(1:8)(p13;q23); t(13;20)(q22;q11.2); t(15;16)(q13;q13). Of 49 embryos tested, karyotyping allowed pre-selection of 13 balanced or normal embryos for transfer back to patients. Both of these methods in combination with FISH analysis will allow a significant improvement in PGD of both maternally and paternally derived translocations.

Program Nr: 412 from the 1999 ASHG Annual Meeting

RNA interference of SMN, Spinal Muscular Atrophy gene, leads to embryonic lethality and to germ cell apoptosis in the nematode C.elegans. *S. Bertrandy[1], S. Lefebvre[1], Y. Kohara[2], A. Munnich[1], D. Thierry-Mieg[3]*. 1) INSERM U393, Hopital Necker Enfants Malades, Paris, France; 2) NIG, Mishima, Japan; 3) CRBM-CNRS, Montpellier, France.

Spinal muscular atrophy (SMA) is a frequent autosomal recessive neuromuscular disorder characterized by degeneration of spinal motoneurons. The disease results from alterations of the Survival Motor Neuron (SMN) gene encoding a 294 amino acid protein of unclear function. SMN protein interacts with RNA and RNA-binding proteins and is involved in spliceosomal snRNP biogenesis and pre-mRNA splicing. Recently, we have shown that SMN proteins from C.elegans and Danio rerio bind RNA in vitro. RNA interference experiments were carried out by injection of SMN ds-RNA in wild type animals that generated a progeny with various defects. Two-days post-injection, there was sudden burst of late embryonic lethality that affects 100% of late progeny, indicating SMN requirement for survival in C.elegans. Furthermore, the day after injection, many healthy adult progeny had gonads with few germ cells and signs of germ cell apoptosis. This defect was completely suppressed when injecting the ced-4 (n1162) mutant involved in the execution of the programmed cell death pathway. This epistasis result indicates that SMN has a protective effect on germ line cells. Interestingly, the ced-4 mutant did not suppress the embryonic or larval lethality. Unlike what seems to happen in the mouse, where SMN inactivation led to massive apoptosis in early embryos, here the cause for lethality is not just ced-4 dependant apoptosis. We shall present in situ RNA hybridization and immunofluorescence data in C.elegans. Preliminary results indicate that this ubiquitous gene is highly expressed in gonads and in developing embryos. In very early embryos, the protein localizes mainly to cytoplasmic granules and later in the nucleus. We would like to know if SMN controls alternative splicing of ced-4 in germ line and if it plays a role in maturation, splicing, protection or transport of specific RNAs (especially maternal). Investigations in C. elegans will help understanding the role of SMN in cellular survival.

Program Nr: 413 from the 1999 ASHG Annual Meeting

XIST expression in embryonal carcinoma cells: a model for X-chromosome inactivation in early human development. *J.C. Chow[1], C.M. Clemson[2], J.B. Lawrence[2], C.J. Brown[1]*. 1) Dept. of Medical Genetics, University of British Columbia, Vancouver, BC, Canada; 2) Dept. of Cell Biology, University of Massachusetts Medical Centre, Worcester, Mass.

X-inactivation results in a cis-limited silencing of most genes on one X in females. Xist is required for the initiation and spread of inactivation and in mouse, prior to inactivation, it is expressed as a low-level unstable transcript from both X's. Upon differentiation, only the inactive X obtains a high level of stable Xist expression--a change which may involve a promoter switch or an antisense transcript. To address whether human XIST has the same differential promoter usage and stability changes early in development, we have analyzed expression in human somatic cells and lines derived from earlier in development, including embryonal carcinoma (EC) lines. Using RT-PCR, we found a low-level stable transcript that initiates ~100bp upstream of the main transcriptional start in male and female somatic cell lines and in a male EC line, but no transcription further upstream. From its expression profile in both male and female somatic cells and its stable nature (T1/2 ~5h), this upstream transcript does not appear to be the human equivalent of the mouse upstream promoter. We also detected expression 18 kb downstream of the published 3'end of XIST and are currently characterizing the transcript to determine its orientation and start site. Using quantitative RT-PCR, XIST levels in female somatic cells were found to be ~2000 molecules/cell. In male and female EC lines, however, XIST levels were significantly lower, 1/100 and 1/10 of normal female somatic levels, respectively. This is due in part to the fact that these lines contain a mixed population of cells as seen by RNA-FISH, with some cells showing a low-level pinpoint XIST signal that colocalises with the X chromosome. We are currently determining the inactivation status of the X-chromosomes present in the EC lines and monitoring changes after induction of differentiation in order to identify cell lines representative of early stages of human development.

Program Nr: 414 from the 1999 ASHG Annual Meeting

Association of the sonic hedgehog receptor patched with caveolin-1. *M.R. Gailani, J. Bukowski, H.J. Karpen.* Dept Pediatrics, Yale Univ, New Haven, CT.

The hedgehog signalling pathway, first described in Drosophilla and conserved in vertebrates, plays an important role in early embryonic patterning and continued control of cell growth. Activation of the pathway results in cancer including basal cell carcinoma of the skin. Sonic hedgehog (verterate homologue, SHH) acts on target cells to increase transcription of several genes including its receptor patched (PTCH). PTCH, a negative regulator of the pathway, associates with a second membrane protein smoothened (SMO), inhibiting its function. When SHH binds to PTCH, SMO can transmit the SHH signal. We hypothesized that the PTCH/SMO receptor complex localizes to the cell membrane and other proteins may be involved in complex formation. To study this, PTCH and SMO cDNAs were linked to the green fluorescent protein cDNA, transfected into COS cells, and examined under confocal microscopy. The majority of PTCH was concentrated in the golgi with some of the protein moving to and from the cell membrane. A 3-D reconstruction of the cell suggested PTCH localized just under the membrane in association with caveolae; small micro-invaginations of the cell membrane. Caveolin-1, the major structural protein of caveolae, concentrates a diverse group of signalling molecules within caveolae, usually in an inactive state. We hypothesized that the SHH receptor complex is sequestered in caveolae through a direct interaction between PTCH and caveolin-1. Co-immunoprecipitation and immunocytochemistry (ICC) studies confirm that the two proteins associate with each other. Similar experiments with SMO and caveolin show no direct interaction, however all three proteins co-localize at the cell membrane. PTCH contains a putative caveolin-1 binding site which directly binds caveolin-1 in GST fusion protein binding experiments. Co-fractionation experiments confirm that caveolin-1 and PTCH are at the cell membrane. The data suggests a model by which the SHH receptor complex is held in an inactive state at a defined region in the cell membrane. Future studies will examine the effect of the SHH protein on this complex. Knowledge of protein interactions in the SHH receptor complex may suggest drug targets and therapeutic interventions aimed at susceptible tumors.

Program Nr: 415 from the 1999 ASHG Annual Meeting

Functional and expression studies of APECED protein and its mouse homologue. *M.K. Halonen[1,2], P. Bjorses[1,2], J.J. Palvimo[3], M. Pelto-Huikko[4], L. Peltonen[5,1], M. Kolmer[1], I. Ulmanen[1]*. 1) Department of Human Molecular Genetics, National Public Health, Helsinki, Finland; 2) Hospital for Children and Adolescence, Helsinki University Hospital, Finland; 3) Department of Physiology, Unversity of Helsinki, Finland; 4) Department of Anatomy, University of Tampere, Finland; 5) Gonda Neuroscience and Genetics Research Center, UCLA, Department of Human Genetics, Los Angeles, California, USA.

Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) is an autoimmune disease with autosomal recessive inheritance. We have cloned the defective gene named AIRE and shown that in transiently transfected mammalian cell lines the APECED protein is mainly located in specks in the cell nucleus. APECED protein harbors two PHD zinc fingers, four LXXLL domains, a SAND domain and an ASS domain suggesting a function in transcription regulation. By immunohistochemistry the human APECED protein appears in immunologically relevant tissues such as the thymus, spleen, lymph nodes and peripheral leukocytes. We have characterized 16 different mutations in APECED patients. Four mutations affecting different domains of the protein were further analyzed by in vitro transient expression to examine the effects of the mutations on the subcellular targeting of the APECED protein. Most of the mutant proteins were not transported to the nucleus. Using transactivation assay we examined the potential of the mutant APECED proteins to activate transcription. For most of the mutants analyzed, the stimulating effect on transcription was totally lost or significantly reduced. We have also cloned the mouse APECED cDNA. The expression pattern of the mouse Aire gene was studied in various transfected cell lines, finding the subcellular localization very similar to the human counterpart. Utilizing antisera against mouse peptides we have studied the tissue distribution of APECED protein in mouse. The mRNA distribution was characterized by in situ hybridization. The results obtained by both methods indicate that mouse APECED protein is expressed in specific thymal cells as well as in other immunological tissues. The mouse Aire gene also exhibits transactivation properties.

Program Nr: 416 from the 1999 ASHG Annual Meeting

Localization of BLM in the PML-nuclear bodies is mediated by a functional domain within its N-terminus. *P. Hu, T. Ye, S.F. Beresten, R. Stan, A.J. van Brabant, N.A. Ellis.* Department of Human Genetics, Memorial Sloan-Kettering Cancer Center, New York, NY, 10021.

Blooms syndrome (BS) is a rare, autosomal recessive disorder characterized by short stature and a predisposition to developing cancers of all types. Cells derived from BS patients exhibit hypermutability and increased chromatid exchange including evaluated sister-chromatid exchange (SCE). BLM, the gene mutated in BS, encodes a DNA helicase that belongs to the RecQ DNA helicase family and stable transfection of BLM can bring to or toward normal the high-SCE phenotype of BS cells. By indirect immunofluorencence, we observed the BLM protein in two predominant patterns: 1) discrete nuclear dots that colocalize with the PML-nuclear bodies (PML-NBs), and 2) diffuse microspeckles. In order to understand the function of BLM in the PML-NBs, we mapped the functional domain in BLM that is required for its PML-NB localization. Ten different mutated BLM constructs were generated in fusion with GFP, including one with the helicase domain (amino acids 674-1114) deleted, four with various deletions from the N-terminus (from amino acid 1 to 568), and five N-terminal segments linked to the nuclear localization signal. Each construct was transiently transfected into HeLa cells, and the cells were immunostained with anti-PML. When normal GFP-BLM was transfected, approximately 50% of the cells exhibit GFP dots and nearly all GFP dots colocalized with the PML-NBs. By transfecting mutant GFP-BLM constructs and counting the percentage of cell with GFP dots and GFP dots in the PML-NBs, we mapped a 300 amino acid region in the N-terminus of BLM that is sufficient to mediate translocation into the PML-NBs. SCE analysis has been carried out on several key constructs after they were permanently transfected into BS cells, and preliminary evidence suggests that cells containing BLM proteins which fail to localize to the PML-NBs have SCE frequencies that are intermediate; that is between normal and vector-transfected BS cells. Helicase assays are now underway. These results will allow us to determine whether localization of BLM in the PML-NBs is required for maintenance of genomic stability.

Program Nr: 417 from the 1999 ASHG Annual Meeting

Structure of the giant *HERC2*gene and identification of multiple partial paralogs. *Y. Ji[1], N.A. Rebert[1], J.M. Joslin[2], M.J. Higgins[3], R.A. Schultz[2], R.D. Nicholls[1]*. 1) Case Western Reserve Univ., Cleveland, OH; 2) Univ. Texas Southwestern Med. Cntr., Dallas, TX; 3) Roswell Park Cancer Inst., Buffalo, NY.

Recombination between chromosomal specific low-copy repeats (duplicons) is the underlying mechanism for several microdeletion syndromes. Recently, we and others identified a chromosome 15 duplicon in the common breakpoint regions of Prader-Willi (PWS) and Angelman syndrome (AS) deletions. We identified a large 15 kb transcript, *HERC2*, as an ancestral gene in this duplicon as well as a family of ~11 *HERC2*-related genes. A rearranged *HERC2* transcript was detected in 1 of 5 PWS/AS deletion patients. Therefore, we proposed that the presence of large blocks of homologous sequence and active transcription of *HERC2*-related duplicons at 15q11 and at 15q13 facilitates recombination and leads to the PWS/AS deletions. *HERC2* encodes a giant protein of 528 kD, with motifs suggesting function as a guanine nucleotide exchange factor and E3 ubiquitin protein ligase. We report here characterization of the *HERC2* gene structure and duplicated segments, and identify a *Drosophila* homolog. A genomic contig of *HERC2* was sequenced, revealing a total of 93 exons spanning ~250 kb, and a strong CpG island promoter. A ribosomal protein L41 processed pseudogene was found in intron 2 of *HERC2*, and a putative VNTR comprised of 27 copies of a ~76-bp repeat occurs in intron 70. Multiple duplicated but rearranged copies of *HERC2* were identified by database analysis. Sequence comparison indicates that *HERC2* has undergone several deletion events to form complex duplicons in 15q11, 15q13 and 16p11. Zoo-blot analysis identified *HERC2* homologs from human to *Drosophila*. Previously, we identified a unique mouse *Herc2* gene with 96% amino acid identity to human HERC2 and showed that mutations of *Herc2* lead to a developmental syndrome, *jdf2*. A partial *Drosophila* homolog was cloned, with amino acid identity of 70% to human HERC2 over 742 residues. Using BAC clones, we are mapping the chromosomal location to establish *Drosophila* as a model organism. These studies, and ongoing protein analyses, further our understanding of the function of *HERC2* in development and putative role in protein trafficking pathways.

Program Nr: 418 from the 1999 ASHG Annual Meeting

The order of intron removal influences the outcome of splice site mutations. *C.D. Kuslich, U. Schwarze, P.H. Byers.* University of Washington, Seattle, WA.

Splice site mutations cause many genetic disorders but it is unclear why identical mutations (e.g. IVSX+1G®A) have different outcomes, i.e. exon skip, intron inclusion or use of cryptic donor sites. We postulated that preferred order of intron splicing dictates the outcome of splice site mutations. To test this hypothesis we determined the order of intron removal in selected regions of three human collagen genes (COL1A1, IVS14-19; COL1A2, IVS19-22, IVS28-31 and IVS41-44; and COL3A1, IVS18-22) and examined the consequences of splice mutations in these areas. We incubated dermal fibroblasts with ActD for 5-40 minutes, isolated nuclear RNA, made cDNA and used intron/exon primer pairs to amplify intron-containing transcripts. Intron primers were labeled with fluors or ^{32}P and the products analyzed after PCR in the linear range by separation on the ABI310™ or PAGE, respectively. In the region examined in COL1A1, intron 16 is removed last in most transcripts. In COL1A2, the introns in the 19-22 domain are removed in reverse order, in 28 - 33 IVS32 is removed early, and in 41 - 44 region IVS42 is removed first in the major and 43 is spliced first in an alternative pathway. In COL3A1 there are alternative pathways in which IVS20 is usually removed late and infrequently before IVS19. Four +1G®A donor site mutations in these genes have different outcomes. In COL1A2 IVS30+1G®A results in exon 30 skip only; IVS32+1G®A skips and uses rare AT/AC terminal dinucleotides; IVS43+1G®A results in intron inclusion (major) and exon 43 skip (minor). In COL3A1, IVS20+1G®A results in rare exon skip, use of cryptic donor sites within the exon or intron, and intron inclusion. In COL1A1, IVS16-2A®G results in exon skipping (rare) and use of a cryptic acceptor site while in COL3A1 IVS19-3T®G results in exon 20 skipping only. These outcomes are all consistent with the following model: donor or acceptor site mutations in rapidly removed introns lead to exon skipping but the same mutations in slowly removed introns lead to use of cryptic sites or whole intron inclusion. Complex outcomes are consistent with multiple splicing pathways. Consequently, knowledge of splice order is essential for correct prediction of splice mutation outcomes. (NIH AR21557).

Program Nr: 419 from the 1999 ASHG Annual Meeting

Rent1, a mammalian *trans*-effector of nonsense-mediated RNA decay, is essential for cellular viability. *S.M. Medghalchi, H.C. Dietz.* HHMI and The Institute of Genetic Medicine, Johns Hopkins Univ School of Medicine, Baltimore, MD.

The ability to detect and degrade transcripts that lack full coding potential is ubiquitous but nonessential in lower eukaryotes, leaving in question the selective pressure for complete evolutionary maintenance of this function. One hypothesis holds that nonsense-mediated RNA decay (NMD) protects the organism by preventing the translation of peptides with dominant negative or deleterious gain-of-function potential. All organisms that are competent for NMD express a structural homologue of *S. cerevisiae* Upf1p. Recent data suggest that rent1, the mammalian counterpart, is essential for NMD in cultured cells. We have now explored the consequence of loss of rent1 and NMD function in vertebrates through targeted gene disruption in murine embryonic stem cells. The targeting event is predicted to delete all functional domains from rent1. Germline transmission of the targeted Rent1 allele was observed and resulting heterozygous (+/-) offspring showed no apparent phenotypic abnormalities. Fibroblasts derived from (+/-) animals showed full efficiency of NMD despite absence of immunodetectable protein from the mutant allele, suggesting that half-normal levels of rent1 can support the pathway. Breeding of (+/-) animals resulted in the expected 1:2 ratio between (+/+) and (+/-) offspring, but (-/-) mice were never observed ($p<0.0002$) documenting that rent1 is essential for embryonic viability. Analysis of staged embryos documented that (-/-) blastocysts are viable in the preimplantation stage but resorb immediately after implantation (3.5 and 6.5 d.p.c., respectively). Isolated day 3.5 p.c. (-/-) embryos emerged from the zona pellucida but the inner cell mass progressively regressed in culture. After 4 days in culture, both the inner cell mass and trophoblasts had completely deteriorated. In contrast, cells derived from (+/+) and (+/-) embryos proliferated and differentiated in culture. These data suggest that NMD is essential for mammalian cellular viability and support a role for the pathway in the regulated expression of critical physiologic transcripts. Ongoing studies using a conditional rescue allele should help to define the role of NMD in cellular metabolism.

Program Nr: 420 from the 1999 ASHG Annual Meeting

Functional genomics of the B-box gene family reveals a possible role in subcellular compartmentalization. *G. Meroni[1], A. Reymond[1], A. Fantozzi[2], S. Cairo[1], D. Riganelli[2], G. Merla[1], E. Zanaria[1], S. Messali[1], L. Luzi[2], A. Guffanti[1], P.G. Pelicci[2], A. Ballabio[1]*. 1) Telethon Inst Genetics & Med, Milan, Italy; 2) European Inst Oncology, Milan, Italy.

The genes belonging to the B-box family have been implicated in a variety of processes. Among them, PML, RFP, and Tif1 acquire oncogenic activity when fused to RAR alpha, RET, and B-raf, respectively. Recently, two additional B-box genes involved in human diseases have been identified, the FMF gene which is mutated in Familial Mediterranean Fever, and the MID1 gene, involved in X-linked Opitz syndrome. These genes contain a RING, two B-boxes, a coiled-coil, and a frequently conserved C-terminal domain. Very little is known about the molecular mechanisms mediating the function of B-box genes and thus we decided to redefine and characterize the entire family using a systematic and comprehensive approach to efficiently move from sequence/structure information to functional knowledge. We identified novel members of the B-box family by a systematic search of the dbEST and we have collected the full-length cDNA of the 15 known and 18 novel B-box genes. To evaluate the possible involvement of the B-box genes in human diseases, we have defined their chromosomal position and analyzed their expression patterns in adult tissues and during development. In addition, we have designed functional assays which allowed us to find that many family members show a strong growth suppression potential and are able to repress transcription. Moreover, we found that most of the B-box proteins are able to homodimerize and to form large multiprotein complexes. The B-box proteins, through their ability to form multiprotein complexes, appear to play an extremely important role in defining different subcellular compartments, both in the nucleus and in the cytoplasm, suggesting a possible involvement in compartimentalizing other proteins. These data will be useful to better understand the role of this class of proteins during normal development and to assess their role in the pathogenesis of tumors and inherited diseases.

Program Nr: 421 from the 1999 ASHG Annual Meeting

Characterization of a novel maternally imprinted human gene and its mouse ortholog located in the PWS region.

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Within the PWS critical region, six imprinted genes which are expressed from the paternal allele only, have been isolated. SNRPN and NDN{JAY97, encode for proteins, IPW is spliced and polyadenylated and has been suggested to play a functional role as a RNA, PAR1 and PAR5 are two uncharacterized transcripts. Deficient mice for Snrpn, Ipw, Zfp127 and Ndnhave been independently created and any biological role cannot be associated to one of these genes in the etiology of the PWS mouse model phenotype. We now report the characterisation of a novel gene, nM, from the PWS region. We have shown that nM is imprinted and expressed from the paternal allele in human brain and fibroblasts.We have characterized and localised its murine ortholog, nS, in the syntenic region to the human PWS region. We demonstrate that nS is also expressed only from the paternal allele in brain. The mouse and human nM orthologous genes encode putative proteins may play a role in the etiology of the PWS phenotype and its mouse model respectively.

Program Nr: 422 from the 1999 ASHG Annual Meeting

A TIGR (or MYOC,GLC1A) Gene Promoter Polymorphism Associates with Steroid-Glaucoma and Evidence for Its Tissue Specific Expression in The Human Trabecular Meshwork Cells. *T.D. Nguyen[1], H. Chen[1], L. Kapp[1], S. Shirato[3], K. Kawasi[2], Y. Kitazawa[2], J.R. Polansky[1]*. 1) Dept. Ophthalmology, Univ California, San Francisco, San Francisco, CA; 2) Dept. Ophthalmology, Gifu University, Gifu 500, Japan; 3) Dept. of Ophthalmology, Univ. of Tokyo, Tokyo 113, Japan.

Since the steroid induction of the TIGR gene is a feature potentially relates to clinical manifestation of steroid-glaucoma patients, we evaluated possible promoter mutation(s) among these patients and analyzed their influence on the promoter functions. Genomic DNAs of 26 patients and 23 normal individuals were collected based on intraocular pressure (IOP) elevation by sustained topical steroid testings for 4 weeks (Armaly MF,1963). A polymorphic sequence of the promoter, namely TIGR.mt11, was found in over 50% of the patients but not found in the normals. Subsequently, a 283 bp promoter region that covers the TIGR.mt11 or TIGR.wt (wild type) sequences was cloned into alkaline phosphatase (AP) reporter gene vectors (SEAP, Clontech, CA.) and we showed that the TIGR.mt11 has more than 5 folds of AP activities over the TIGR.wt construct by transfection studies using transformed human trabecular meshwork (TM) cells. In similar studies for Hela and COS-7 cells, we found a significantly reduced AP activity compared to the TM cells. A similar AP activity was found for the three cell lines when a 1.4 kb promoter region was used suggesting that the 283 bp region is a target of TM tissue specific binding. Results obtained from gel shift assays for glucocorticoid treated and untreated TM and Hela cells have provided evidence for such a DNA binding protein. The TIGR.mt11 sequence variant and its effects on TIGR gene expression suggest possible association of the TIGR gene and steroid-glaucoma. Future studies of the mechanism of TIGR.mt11 mutation on TIGR gene expression, and characterization of the TM tissue specific factor(s) might be relevant for understanding genetics/pathogenesis of steroid-glaucoma, and possibly other forms of glaucoma. Supported by The Glaucoma Research Foundation, That Man May See, and InSite Vision, Inc..

Program Nr: 423 from the 1999 ASHG Annual Meeting

How does an A-to-G splice donor site mutation at position +3 result in aberrant splicing? A lesson learned from a mutation in the *COLQ* gene. *K. Ohno, J.M. Brengman, A.G. Engel.* Department of Neurology, Mayo Clinic, Rochester, MN.

Congenital endplate acetylcholinesterase (AChE) deficiency, the cause of a disabling myasthenic syndrome, arises from defects in the *COLQ* gene encoding the triple helical collagenic tail subunit of AChE that anchors catalytic subunits of AChE to the synaptic basal lamina. We recently cloned human *COLQ* cDNA, determined the genomic structure of *COLQ*, and identified 6 recessive truncation mutations in 6 patients (*PNAS* 95: 9654, 1998). We here report two novel disease-associated *COLQ* mutations: an A-to-G substitution at position +3 of intron 16 (IVS16+3A®G) and R315X. Family analysis shows that the mutations are heteroallelic and recessive. Because both A and G are consensus nucleotides at the +3 position of splice donor sites, we constructed a minigene spanning *COLQ* exons 15 to 17 and harboring IVS16+3A®G, and analyzed pre-mRNA splicing in COS cells. This revealed that IVS16+3A®G causes skipping of exon 16. The mutant splice donor site of intron 16 harbors five discordant nucleotides at -3, -2, +3, +4, and +6 that do not base-pair with U1 snRNA, the molecule responsible for splice donor site recognition. Using additional constructs of the minigene harboring IVS16+3A®G, we also show that a nucleotide complementary to U1 snRNA either at +4 or +6 restores normal splicing, whereas a complementary nucleotide at -3 or -2 has no or little beneficial effect on splicing. Consistent with a role of residues +4 and +6 in splicing, analysis of 1801 human native splice donor sites reveals that presence of a G nucleotide at +3 is associated with preferential usage of nucleotides concordant to U1 snRNA at positions +4 to +6. Analysis of 11 disease-associated IVS+3A®G mutations indicates that on the average 2 of 3 nucleotides at positions +4 to +6 fail to base-pair, and that the nucleotide at +4 never base-pairs, with U1 snRNA. We conclude that with G at intron position +3, normal splicing generally depends on concordance of intronic residues at +4 to +6 with U1 snRNA, but additional *cis*-acting elements may also participate in assuring fidelity of splicing.

Program Nr: 424 from the 1999 ASHG Annual Meeting

Gene-specific versus regional control of X inactivation: status of murine X-linked genes. *K. Tsuchiya, R.A. Conlon, H.F. Willard.* Case Western Reserve Univ. Cleveland, OH.

X inactivation results in transcriptional silencing of most, but not all genes on the inactive X chromosome. The factors that are responsible for the expression of a gene from the inactive X are unknown. Control of expression of X-linked genes may occur at the level of individual genes, at the level of chromosomal domains, or both. The clustering of six genes that escape inactivation in a 370 Kb region of human Xp11.2 indicates that there is at least one coordinately regulated domain on the human inactive X. To determine if this domain is present on the mouse X chromosome, we have characterized the mouse homologues of genes mapping in and adjacent to the Xp11.2 escape domain. We have determined that the mouse Dxs1008E, Smcx, Sb1.8, Ads9, and Ib772 genes are all present on the same 600 Kb mouse YAC, in the same relative order as the human genes. While human SMCX and mouse Smcx genes are known to escape inactivation, the mouse Sb1.8 gene is subject to inactivation, in contrast to human SB1.8. To extend these previous findings, we have now characterized the murine Dxs1008, Ads9, and Ib772 genes. Polymorphisms were identified and expression was tested by restriction enzyme digestion of RT-PCR products from two non-randomly inactivated F1 females resulting from a cross between t(X;16) carrier females and M. castaneus males. All three genes were subject to inactivation. The observation that Smcx is the only mouse gene in this region so far that escapes inactivation suggests that specific sequences in or near this gene may influence its expression from the inactive X. To test this hypothesis, we have made a construct consisting of a Betageo reporter under the control of 2.7 Kb of the mouse Smcx promoter. We have targeted this construct to Hprt, a locus on the X chromosome that is subject to inactivation. Six chimeric (80-100%) male mice have been generated and are being bred for germline transmission of the construct. We will then assess the X inactivation status of the reporter gene in hemizygous females by assaying lacZ expression, to test whether the Smcx promoter can confer the ability to escape inactivation in a different location on the X chromosome.

Program Nr: 425 from the 1999 ASHG Annual Meeting

The product of *Dazl*, a *DAZ* (Deleted in AZoospermia)-like gene, is associated with actively translating polyribosomes. *S. Tsui[1], T. Dai[1], E. Salido[2], S. Warren[3], P. Yen[1]*. 1) Medical Genetics, Harbor-UCLA Medical Center, Torrance, CA; 2) Pathology, University of La Laguna, Spain; 3) Howard Hughes Medical Institute, Emory University, Atlanta, Georgia.

The human *DAZ* gene family on the Y chromosome is deleted in about 10% of infertile males with idiopathic azoospermia. *DAZ* homologues are present on the Y chromosomes of great apes and old-world monkeys only. However, there is an autosomal *DAZL* (DAZ-like) gene that is present in all mammals examined. The DAZL and DAZ proteins, which are expressed in germ-cells only, contain an RNA recognition motif and either a single copy (as in DAZL) or multiple copies (as in DAZ) of a DAZ repeat. A role for DAZ and DAZL in spermatogenesis is supported by their homology to a *Drosophila* male infertility protein Boule, and the sterility of *Dazl* knock-out mice. The biological pathway in which these proteins act is unknown. Mouse Dazl was shown by immunostaining to be present mainly in the cytoplasm. We present data demonstrating that DAZ and DAZL bind to RNA polymers, preferentially poly U and poly G, *in vitro*. We also used an anti-DAZL antibody to trace Dazl in mouse testicular extracts and subcellular fractions. The majority of Dazl was found in the post-mitochondrial supernatant (PMS). On sucrose gradients, most Dazl co-migrated with polyribosomes, similar to the fragile X mental retardation protein Fmrp. Treatment of PMS with 30 mM EDTA, which dissociates polysomes into ribosomal subunits, or with RNase A, which breaks the polysomes into monosomes, caused a significant reduction in the sedimentation rate of Dazl, further supporting its association with polysomes. Additional studies indicated that Dazl was associated with polysomes through its binding to poly(A) RNA, which was affected by RNase and NaOH but not EDTA treatment. Our results implicate Dazl in translational regulation in germ cells.

Program Nr: 426 from the 1999 ASHG Annual Meeting

The cis-preference model of L1 retrotransposition. *W. Wei[1], E. Ostertag[2], H.H. Kazazian[2], J.V. Moran[1].* 1) Human Genetics, Univ. of Michigan Med. Sch., Ann Arbor, MI; 2) Genetics, Univ. of Pennsylvania Sch. of Med., Philadelphia, PA.

Mutagenic L1 insertions have resulted in a variety of diseases. These insertions appear to be derived from retrotransposition-competent progenitor L1s (RC-L1s), containing two intact open reading frames (ORF1 and ORF2). Thus, we proposed that the L1-encoded proteins preferentially function to both bind and retrotranspose the RNA from which they are translated. Here, we present evidence in strong support of this cis-preference model.

Previously, we developed an assay to monitor L1 retrotransposition in HeLa cells. We tagged L1s with an indicator gene (mneoI) designed to detect retrotransposition events and showed that about 30-60 RC-L1s are present in the genome. We also found that point mutations (PM-L1s) in the ORF1 or ORF2-encoded proteins abolish retrotransposition. Here, we used a transient assay to monitor L1 retrotransposition in a rapid, quantitative manner. We first found that the ORF1-encoded protein cannot function in trans to retrotranspose an RNA consisting of L1 ORF2 and mneoI. Thus, L1 retrotransposition requires that ORF1 and ORF2 are encoded by the same L1 RNA molecule.

We next found that PM-L1s containing mneoI are complemented inefficiently when co-transfected into HeLa cells with different RC-L1s (L1.3 or RP). Four ORF1 mutants are complemented at very low levels, whereas six ORF2 mutants are complemented at somewhat higher levels (about 0.01% and 1% the frequency of L1.3 retrotransposition, respectively). Moreover, L1.3 or RP rarely complements an RNA containing only L1 ORF2 and mneoI. Thus, the presence of a functional ORF1 protein in the PM-L1s is required for low level complementation. We further confirmed that the PM-L1s are not dominant negative mutations because their expression does not inhibit the retrotransposition of tagged RC-L1s. Together, our data show that the proteins encoded by RC-L1s preferentially function in cis to retrotranspose the RNA from which they are translated, but can function in trans at low levels to mobilize other L1 RNAs.

Program Nr: 427 from the 1999 ASHG Annual Meeting

Hes-1 binds to an E box within intron 1 (IVS I) of the human acid alpha-glucosidase gene and acts as a transcriptional repressor. *B. Yan[1], N. Raben[1], K. Nagaraju[1], R. Nichols[2], P. Plotz[1]*. 1) ARB, NIAMS/NIH, Bethesda, MD; 2) Department of Medicine, Dartmouth Medical School, Hanover, NH.

Glycogenosis type II, a severe, recessively-inherited myopathy and cardiomyopathy (Pompe syndrome, acid maltase deficiency), is caused by deficiency of the lysosomal enzyme acid alpha-glucosidase (GAA), leading to abnormal glycogen accumulation in lysosomes. Although the GAA gene has been cloned and sequenced, very little is known about its regulation and expression. In previous studies, we have demonstrated that IVS I(~2.7kb) contains a negative regulatory element. In this study, reporter gene constructs and transient transfection assays were employed to localize the putative regulatory element. A series of deletion fragments of IVS I were cloned in both orientations upstream of bacterial chloramphenicol acetytransferase (CAT) gene under control of the thymidine kinase (TK) gene promoter and were assayed in Hep G2 cells. The negative regulatory element was localized to a 90bp fragment within IVS I which repressed the activity of the TK promoter by ~ 50% in both orientations. Footprinting analysis revealed a protected region of 23bp containing a potential YY1 binding site and two E boxes, CATCTG and CACGCG. The latter belongs to E box class C and is a specific binding site for Hes-1, which is in the Notch signal transduction pathway. Further transfection assay confirmed the repressive effect of the protected region, and gel-shift assay revealed that two proteins specifically bind to it. Super-shift assay with antibodies confirmed that the binding proteins are transcriptional factors Hes-1 and YY1. Hes-1, a mammalian homologue of Drosophila Hairy, is a basic helix-loop-helix (bHLH) factor and functions as a developmental transcriptional repressor in both invertebrates and vertebrates. We have demonstrated here that Hes-1 plays a role in the regulation and expression of a disease- related human housekeeping gene.

Program Nr: 428 from the 1999 ASHG Annual Meeting

Jameslink: An innovative touch-screen kiosk system for tailored cancer risk assessment and notification. *J.S. Graham, T.L. Bradley, H. Hampel, J.A. Westman, F.A. Wright.* Human Cancer Genetics Program-Comprehensive Cancer Center, The Ohio State University, Columbus, OH.,USA.

It has been shown that healthcare professionals rarely ascertain and record family history data, recognize specific cancer susceptibility syndromes, and refer appropriate families for genetic counseling. In response to this need, our program has developed a model touch-screen kiosk that systematically captures personal and family cancer history data, and allows for manual evaluation of the data and communication of results to the kiosk user. Fields collected include any cancer diagnosis in the user and first- or second-degree relatives, including ages of onset, multiple primaries, and maternal and paternal ethnicity. Family histories are classified according to criteria derived from peer-reviewed literature. The ultimate goals of the project are to educate the user about the family's likely cancer pattern (sporadic, familial, or hereditary) and corresponding surveillance or risk-intervention strategies and accrue appropriate families to pertinent research trials. Users receive a risk assessment letter regarding cancer risk level (general population, increased risk, significant risk) and the ACS screening guidelines. When appropriate, enhanced guidelines are provided (increased site-specific surveillance and/or genetic counseling). Evaluations on 663 individuals have been completed. The risk data are as follows: general population, 57%; increased surveillance, 17%; hereditary, 11%. Of those surveys evaluated, 11% did not contain sufficient data to score. The remaining 3% of the sample include those surveys demonstrating an early age of onset in the absence of family history. Studies are ongoing to evaluate user preferences, data accuracy and validity, kiosk methodology, and adequacy of information with regard to risk assessment. Video recording and focus group evaluations are planned. Pilot data have demonstrated that the Jameslink system is very effective at recording data required for risk assessment, but also provides a means for translating that data into a tailored message that educates the entire family about cancer risk and intervention options.

Program Nr: 429 from the 1999 ASHG Annual Meeting

How Confident are Health Professionals in Providing Genetic Services? The Role of Genetics Education. *E.V. Lapham[1], J.O. Weiss[2], C. Kozma[1], J.L. Benkendorf[1], M.A. Wilson[2].* 1) Georgetown Univ Medical Center Washington, DC; 2) Alliance of Genetic Support Groups, Washington, DC.

With the increase in genetic information, the exponentially increasing need for genetic services, and the relatively few genetics professionals, a wide range of health professionals are providing genetic services in hospitals, clinics, geriatric facilities, schools and other places. Their services include conducting intake interviews, taking family and medical histories, providing various kinds of therapies and counseling, interpreting assessment results, participating in case conferences, providing case management, and referring clients to community based resources. This was verified in a 1998 survey of 3600 members (57% response) of six professional associations: dietitians (ADA), occupational therapists (AOTA), physical therapists (APTA), psychologists (APA), speech-language pathologists and audiologists (ASHA), and social workers (NASW). The study showed that 70% discussed the genetic component of problems with at least a few of their patients, 19% made referrals for genetic counseling, 15% referred for genetic testing and 29% provided counseling about genetic concerns. How confident are these health professionals in providing genetic services? While 67% take family histories, only 1 in 4 feel confident in eliciting genetic information. Although most social workers (87%) and psychologists (79%) provided psychosocial counseling, only 26% and 29% respectively have confidence in counseling clients making decisions about genetic testing. Less than half of the social workers (45%) and psychologists (49%) feel confident in helping patients cope with a newly diagnosed genetic disorder or test result. How much formal education in genetics have the health professionals had? Overall, 21% had one or more courses in genetics, 44% had genetic content in course work and 33% had none. High confidence is correlated to amount of genetics education, independent of overall education ($p<.05$) for most services. The need for education to incorporate genetics into the practices and teaching of these professionals is vital if they are to confidently meet the genetic needs of their patients.

Program Nr: 430 from the 1999 ASHG Annual Meeting

Cancer Genetic Counseling and Testing by Telemedicine. *T.M. Diamond, R. Sutphen.* H Lee Moffitt Lifetime CA Scr, Tampa, FL.

Telemedicine is the practice of medicine at a distance using videoconferencing. This technology is increasing in importance due to reduced equipment costs and the potential to deliver medical advice and health care to areas less well served by traditional services. Since cancer genetic counseling relies primarily on detailed analysis of family history information and medical records rather than physical findings, it would seem to be an ideal candidate for the use of telemedicine. Also, since information provided during cancer genetic counseling is often relevant to multiple family members telemedicine may offer a method for simultaneous delivery of genetic information to family members who are geographically distant. However, little published experience exists regarding genetic counseling via telemedicine, and it is possible that the sensitive nature of these sessions may make telemedicine less than optimal for the delivery of these services. We are evaluating the use of telemedicine as a method to deliver cancer genetic counseling and testing services. Individuals presenting for cancer genetic counseling are asked to participate in the telemedicine study and are randomly assigned to receive counseling in a standard face-to-face meeting or by telemedicine. Study participants who are eligible for and elect to proceed with genetic testing receive their results and post-test counseling by the opposite delivery method, i.e. participants who had pre-test counseling by telemedicine have their disclosure in a face-to-face meeting and vice versa. Questionnaire instruments are used to: 1)examine differences between indivduals who elect to participate versus those who do not, 2) evaluate the participants' pre- and post-test genetic counseling experience, and 3) evaluate the geneticist's pre- and post-test counseling experience. We present preliminary outcomes from this study.

Program Nr: 431 from the 1999 ASHG Annual Meeting

Beliefs and Risk Perceptions regarding Prostate Cancer and its Heredity. *N.H. Arar[1], M. Sarosdy[2], I. Thompson[2,3], D. Troyer[4], L. Hunt[5], R. Plaetke[1].* 1) Div of Nephrology, Univ Texas Health Sci Ctr (UTHSCSA), SA, TX; 2) Div of Urology, UTHSCSA; 3) Div of Urology, Brooke Army Medical Center, SA, TX; 4) Dep of Pathology, UTHSCSA; 5) Dep of Pediatrics, UTHSCSA.

Five to 10% of prostate cancer (CaP) cases have a family history that suggests a genetic basis of CaP. We performed a medical anthropological study based on qualitative methods to investigate CaP patients and their relatives (1)beliefs of the causes and symptoms of CaP (2)opinions concerning the hereditary aspects of CaP, and (3)attitudes towards screening of CaP. Participants were recruited from 25 families enrolled in a CaP family study (on average 4.0 CaP cases per family). Applying open-ended and ethnographic interview techniques, we performed 16 semi-structured interviews. All interviews were tape recorded and transcribed. 13 interviews have already been content-analyzed. Patients were on average 67, the spouse was 68, the son 48 and the daughter 31 years old. Eight participants were Caucasians, 4 African-Americans and 1 Mexican-American. 6/10 patients had at least college education and 7/10 had retired. All the patients and their relatives were aware that CaP runs in their family. 8/10 patients and all relatives believed that CaP in their family is caused by heredity and can progress without any obvious symptoms. However, having a family history of CaP was not perceived as an important risk factor for the male members. Consequently, neither patients (before getting the disease) nor the son had taken any preventive measures to reduce their risk. This finding suggests that awareness of the clustering of CaP in families is not sufficient to encourage (healthy) male relatives to seek early screening. Health planners and genetic counselors should pay special attention to this matter. We will present :the rationale for using a qualitative design, examples of open-ended questions and quotes regarding participants' beliefs, and a summary of the sociodemographic factors and its correlation analysis with participants' beliefs. This research was funded by NIH HG00191/ELSI and the San Antonio Area Foundation (SAAF). The Ascertainment of the CaP families was funded by grants from SAAF and NCI (CA 70183).

Program Nr: 432 from the 1999 ASHG Annual Meeting

Attitudes about hereditary breast cancer among diverse breast cancer survivors. *W.F. Cohn[1], G. Fraser[2], S.M. Jones[3], S. Miesfeldt[4].* 1) Health Evaluation Sciences; 2) Anthropology; 3) Cancer Center; 4) Hematology/Oncology, University of Virginia, Charlottesville, VA 22908.

Much is unknown about the breast cancer genetic counseling and risk assessment process. Until the factors involved in a woman's understanding of the cause and course of hereditary breast cancer (HBC) are known, the clinical utility of major basic scientific advances in this area will not be fully realized. Purpose: To examine the attitudes and beliefs of genetically endangered women from diverse backgrounds concerning the etiology, course and management of HBC. Methods: All women with breast cancer diagnosed < age 50 years and entered in the Virginia Cancer Registry (VCR) in 1995 were eligible for inclusion. Thirty-one hospitals, statewide, entering 370 eligible patients in the VCR, participated in this project. Potential participants received a family history questionnaire and an informed consent document. Responses were received from 132 women: 45 were determined to be at-risk for HBC based on their personal and family histories. Eighteen women completed an in-depth qualitative interview, based on an interview guide, which addressed attitudes and beliefs related to the cause and course of HBC. All interviews were audiotaped and transcribed. Participants included 14 European-Americans, 3 African-Americans and 1 Native-American from throughout the state. Results: Interviews were content analyzed by two investigators using an emergent category system. Analysis of the interviews revealed thirteen major emergent categories relating to the attitudes, knowledge, and beliefs of study participants regarding the cause and course of HBC. Included in these were: factors related to causation, including impact of family history and multifactorial causation; disease risk expectation for self/family; risk reduction strategies; impact of genetic susceptibility on risk management and cancer treatment decisions; information sources, needs and comprehensibility; genetic "blame". These data provide those directly involved with the care of women genetically endangered to develop breast cancer a better understanding of the fears, myths and beliefs that surround this disease.

Program Nr: 433 from the 1999 ASHG Annual Meeting

Genetics education for different audiences: strategies developed by a pharmaceutical company. *E.A. Balkite, P.K. Manasco, R.P. Sutton.* Genetics Directorate, Glaxo Wellcome Research & Dev, Res Triangle Pk, NC.

Recognizing the increasingly important role genetics is playing in drug development, basic laboratory research, and clinical trials, Glaxo Wellcome formed a Genetics Education Team (GenED) in January, 1997 to respond to its education needs. The team's responsibility is to oversee the design and delivery of a comprehensive and prioritized education and training program in genetics for over 60,000 employees worldwide with various levels of knowledge in genetics. GenED consists of an international team of trainers, scientists and communicators from across the company--including Clinical Genetics, Research, Corporate Affairs, Human Resources, Sales Training, and International Medical Education and Training. The team members developed a core genetics curriculum with additional modules in ethics, clinical trials, and SNPs. GenED also surveyed employees to determine how they wanted to learn about genetics. As a result, the program addresses the different levels of understanding needed to educate scientists and non-scientists alike, as well as the preferred way to learn. Education and learning occur via printed materials, audiotapes, videotapes, Intra and Internet web sites, and face-to-face presentations depending on the target audience. Surveys conducted in May,1999 have demonstrated increased knowledge in some areas and identified needs in others for continuing education and training in basic genetics and pharmacogenetics.

Program Nr: 434 from the 1999 ASHG Annual Meeting

Pooling for Mutation Detection. *C.I. Amos.* Dept Epidemiology, UT MD Anderson Cancer Ctr HMB, Houston, TX.

Mutation detection through end-to-end sequencing of genes predisposing for familial colon cancer or breast cancer has been an expensive proposition because, for each familial cancer, several loci, each consisting of a large number of nucleotide bases must be considered (e.g. for breast cancer, BRCA1 or BRCA2 cause similar phenotypes and so each locus must be subjected to analysis). Here, I present a statistical approach for optimally constructing small pools of samples. The optimal pooling strategy depends upon the frequency of mutations per amplified exon or other unit in a population and is constrained by the technical capacity to detect mutations. Statistical considerations show that provided the mutation frequency per exon is less than 1%, pooling of samples results in a substantial decrease in the total number of samples that need to be assayed in order to detect mutations. Optimal pool sizes and the percent of original number of tests is given below:

Mutation Frequency	1%	0.5%	0.1%	0.05%
Optimal Pool Size	4	5	7	10
Percent of Tests	41%	32%	19%	15%

Even without using an optimal pooling strategy, a significant reduction in cost can be effected by pooling. For example, if the limit of resolution for pooling is 5 samples, less than 25% of the total number of tests need to be performed if the mutation frequency per exon is less than .1%. These findings underscore the potential value of sample pooling to control the costs associated with mutation detection.

Program Nr: 435 from the 1999 ASHG Annual Meeting

TNF polymorphisms in siblings with late-onset Alzheimer disease - The NIMH Genetics Initiative AD Study Group. *J.S. Collins[1], R.T. Perry[1], B. Watson, Jr.[1], L.E. Harrell[1], R.T. Acton[1], D. Blacker[2], M.S. Albert[2], R. Tanzi[2], M.G. McInnis[3], S.S. Bassett[3], R.C.P. Go[1]*. 1) Univ Alabama at Birmingham; 2) Massachusetts General Hospital, Boston; 3) Johns Hopkins University, Baltimore, MD.

One suggestive region found during a collaborative genome wide scan for Alzheimer disease (AD) genes in 266 late-onset families, is a 20 cM area located in the HLA region at chromosome 6p21.3. Some of the most significant findings were near the location of the tumor necrosis factor (TNF) gene. TNF is a pro-inflammatory cytokine, which may also assist in limiting the extent and duration of inflammation. Inflammation could be involved in the pathogenesis of AD as plaques may upregulate pro-inflammatory cytokines. Anti-inflammatory drugs have also been shown to delay and prevent the onset of AD.

A G®A polymorphism at -308 of the TNF promoter region has been previously implicated in autoimmune and infectious diseases. This polymorphism and a second polymorphism, a G®A transition at position -238 of the TNF promoter region, were typed in 145 AD families consisting of 558 affected and unaffected siblings. The microsatellite TNFa, located 3.5 kB from the 3' end of TNF, was also typed in these families. These typings were combined to form haplotypes for this region and the haplotype 2-1-2, respectively, was found to be significantly associated with AD with a p-value of 0.003, using the sibship disequilibrim test. The TNFa2 allele, which has been previously associated with a higher secretion of TNF, was also significantly associated (p=0.04) with AD in these families.

Program Nr: 436 from the 1999 ASHG Annual Meeting

Polynesian origins: new insights from the Y-chromosome. *R. Deka[1], B. Su[2], P. Underhill[3], N. Saha[1], S.T. McGarvey[4], J. Chou[2], P. Oefner[3], M.D. Shriver[1], R. Chakraborty[2], J. Martinson[5], L. Jin[2].* 1) Dept Environmental Health, Univ Cincinnati, Cincinnati, OH; 2) Human Genetics Center, Univ Texas Health Science Center, Houston, TX; 3) Dept Genetics, Stanford University, CA; 4) Dept Medicine, Brown University, Providence, RI; 5) Dept Biological Anthropology, Oxford University, Oxford, England.

The question surrounding the colonization of Polynesia has remained controversial. Two hypotheses, one postulating Taiwan as the putative homeland and the other assering a Melanesian origin, have received much attention. The former, dubbed as the 'express train' model, based on linguistic and archeological evidence, has received support from recent mitochondrial DNA data. In this work, we present new data based on 19 biallelic markers on the Y-chromosome in a sample of 551 individuals from 36 populations living in East Asia, Southeast Asia, Taiwan, Micronesia, Melanesia and Polynesia. Surprisingly, nearly none of the Taiwanese Y-chromosome haplotypes was found in Micronesia and Polynesia. Likewise, a Melanesian-specific haplotype was not found in Polynesia. However, all of the Polynesian, Micronesian and Taiwanese haplotypes are present in the extant Southeast Asian populations. Evidently, Y-chromosome data do not lend support to either of the prevailing hypothesis. Genetic divergence between the Taiwanese and the Micronesian/Polynesian populations is twice that between the Southeast Asians and either of these two groups of island populations. We postulate that Southeast Asia provided genetic source for two independent migrations, one toward Taiwan and the other toward Polynesia. (Supported by grants from the NIH, NSF and the Chinese National Natural Science Foundation).

Program Nr: 437 from the 1999 ASHG Annual Meeting

Genomic control for association studies. *B. Devlin[1], K. Roeder[2].* 1) Department of Psychiatry, Western Psychiatric Inst & Cli, Pittsburgh, PA; 2) Department of Statistics, Carnegie Mellon University, Pittsburgh, PA.

A dense set of Single Nucleotide Polymorphisms (SNP) covering the genome and an efficient method to assess SNP genotypes are expected to be available in the near future. An outstanding question is how to use these technologies efficiently to identify genes affecting liability to complex disorders. To achieve this goal, we propose a statistical method that has several optimal properties: it can be used with case-control data and yet, like family-based designs, control for population heterogeneity; it is insensitive to the usual violations of model assumptions, such as cases failing to be strictly independent; and, by using Bayesian outlier methods, it circumvents the need for Bonferroni correction for multiple tests, leading to better performance in many settings while still constraining risk for false positives. A simpler, frequentist method also springs naturally from this work. The performance of our ``genomic control'' method is evaluated by simulations of an association scan (100,000 SNP) using case-contol data drawn from either essentially homogeneous or quite heterogeneous populations. The false postive rate is small for both kinds of samples, but power is reduced for the latter.

Program Nr: 438 from the 1999 ASHG Annual Meeting

SNPs, Linkage Disequilibrium, Shared Chromosomal Segments, and Genome-Wide Association Mapping. *P.J. Donnelly, C. Wiuf.* Dept Statistics, Univ Oxford, Oxford, England.

When it first arises, a particular mutation will be on a unique haplotypic background. Assuming that the mutation arose only once, chromosomes in the current population which carry it will all share a region, IBD, around the mutation. We present a theoretical analysis, based on population genetics modelling and simulation, of the likely size of this shared region and of the way in which it depends on the population frequency of the mutation, and the demographic history of the population. These results are contrasted with those for regions shared by chance in arbitrary samples from the population. We discuss consequences for patterns of linkage disequilibrium and the required density of proposed SNP maps, and for their use in the design and analysis of genome wide, association-based, studies for genes involved in common, complex, disorders. Our work exploits recent exact results on the structure of conditioned coalescents -- processes which describe the genealogical history of a sample of chromosomes under the assumption that a particular subset of the chromosomes carry a mutation assumed to have arisen uniquely.

Program Nr: 439 from the 1999 ASHG Annual Meeting

Haplotype Analyses in Case-Control Populations: Factors Which Influence Accuracy of Haplotype Estimation.

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Linkage disequilibrium and haplotype analyses have been shown to be effective tools in fine-mapping and candidate gene analyses. However, these methods require determination of phase, which is often not feasible due to unavailability of family members, ancestral information, or cost of laboratory analyses. Accurate estimation of haplotype frequencies within case and control populations, and/or designation of particular haplotypes in ambiguous heterozygote individuals would enable a broader use of linkage disequilibrium analyses in diploid populations, specifically in the commonly used case-control design. We have modified haplotype frequency estimation via the EM algorithm to improve the computational efficiency for analysis of SNPs. We present simulation results demonstrating the overall high level of accuracy of our program, showing that for over 90% of all scenarios simulated, the frequency estimates are within 1% of the generating values. We provide suggestions for programming options including minimum number of restarts, maximum iterations, and maximum convergence criteria that allow for minimal estimation bias. We also show the relative magnitude and direction of influence on accuracy for several factors, including sample size, proportion of ambiguous individuals/heterozygous loci, presence of HWE, underlying haplotype and allele frequencies, number of loci in haplotype, and level of linkage disequilibrium in the area. We also note that most the factors exhibiting even modest influence on accuracy can be assessed directly from the data set of interest.

Program Nr: 440 from the 1999 ASHG Annual Meeting

Evidence for a second major gene for APO-A1 beyond the AI-CIII-AIV gene cluster: The NHLBI Family Heart Study (FHS). *M. Feitosa[1], I. Borecki[1], S. Hunt[2], H. Coon[2], C. Ellison[3], D. Arnett[4], G. Heiss[5], J. Eckfeldt[4], M. Province[1]*. 1) Washington Univ., St. Louis, MO; 2) Univ. Utah, Salt Lake City, UT; 3) Boston Univ., Boston, MA; 4) Univ. Minnesota, Minneapolis, MN; 5) Univ. North Carolina, Chapel Hill, NC USA.

Previous studies have shown that elevated plasma levels of apolipoprotein B (APO-B) and depressed plasma levels of apolipoprotein A1 (APO-A1) are associated with increased risk of cardiovascular disease and premature atherosclerosis. While familial resemblance for APO-A1 and APO-B has been reported, the results on the inheritance pattern are conflicting. Some studies have shown an effect of the apo AI-CIII-AIV gene cluster on these apolipoproteins. With the aim of addressing these issues, complex segregation analysis was applied to a sample of 697 individuals belonging to 436 nuclear families from the FHS. The phenotypes were adjusted for the effects of age, BMI, waist to hip ratio, waist circumference, subscapular to triceps ratio, smoking, alcohol consumption, caloric intake, physical activity and energy expenditure, and apo AI-CIII-AIV genotypes. For APO-B, there was no suggestion of a major gene. The Mendelian hypothesis was rejected (p=0.02) and there was no transmission of the major effect (p=0.45). For APO-A1, without adjusting for AI-CIII-AIV genotype, Mendelian transmission fit the data (p = 0.80), however the no-transmission model also was not rejected, yielding an inconclusive result. However, after adjusting for apo AI-CIII-AIV ($p < 0.001$), there was strong evidence for a major gene while the no transmission model was rejected (p= 0.00009). The gene accounted for 41% of the variance, with an allele frequency of 0.51, with an additional multifactorial component accounting for 25% of the variance. This suggests that there may be a second major gene aside from the apo AI-CIII-AIV locus which influences APO-A1 plasma levels.

Program Nr: 441 from the 1999 ASHG Annual Meeting

Complete sequence analysis of the human apolipoprotein E locus reveals previously undetected heterogeneity among e2, e3 and e4 alleles. *S.M. Fullerton[1], A.G. Clark[1], K.M. Weiss[1], S.L. Taylor[2], D.A. Nickerson[2], J. Stengård[3], E. Boerwinkle[4], C.F. Sing[5]*. 1) Departments of Anthropology and Biology, Pennsylvania State University, University Park, PA; 2) Department of Molecular Biotechnology, University of Washington, Seattle, WA; 3) National Public Health Institute, Department of Epidemiology and Health Promotion, Helsinki, Finland; 4) Human Genetics Center, University of Texas Health Science Center, Houston, TX; 5) Department of Human Genetics, University of Michigan Medical School, Ann Arbor, MI.

As part of our ongoing study of genetic contributions to CVD risk, we have analyzed DNA sequence variation at the apolipoprotein E locus (*APOE*) in 96 individuals from four populations (African-Americans from Jackson, MS, non-Hispanic whites from Rochester, MN, Finns from North Karelia, Finland, and Mayans from Campeche, Mexico). Within the 5.5 kb region surveyed we identified 23 variable sites (22 SNPs and 1 indel), including two nonsynonymous SNPs that define the classic e2, e3, and e4 protein alleles. The observed sites segregate as 25 distinct haplotypes and comprise an e2 clade, an e4 clade, and a pair of divergent e3 clades. The root haplotype, identified via human-chimpanzee sequence comparison, falls in the e4 clade, suggesting that the e4 allele is ancestral to the other major alleles at this locus. Interestingly, this allele (which makes up only 13.5% of the chromosomes surveyed here) is now known to be associated with elevated lipid levels and Alzheimer's Disease risk. The most common allelic class, e3 (79.7%), is derived from the e4 clade and is split into two major lineages, from one of which the e2 alleles (6.8%) are derived. Heterogeneity within the three main allelic classes is only partially explained by within-lineage mutational divergence. Instead, substantial recurrent mutation and/or interallelic recombination, often involving sites in the 5' regulatory region of the gene, generate extensive site homoplasy and haplotype diversity.

Supported by NHLBI grants HL39107, HL58238, HL58239, and HL58240.

Program Nr: 442 from the 1999 ASHG Annual Meeting

Familial aggregation of QT interval variability in a general population: results from the NHLBI Family Heart Study. *Y. Hong[1], P.M. Rautaharju[2], P.N. Hopkins[3], D.K. Arnett[4], L. Djoussé[5], J.S. Pankow[5], D.C. Rao[1], M.A. Province[1]*. 1) Div Biostatistics, Washington Univ Sch Medicine, St Louis, MO; 2) Wake Forest Univ., Winston-Salem, NC; 3) Univ. of Utah, Salt Lake City, UT; 4) Univ. of Minnesota, Minneapolis, MN; 5) Boston Univ., Boston, MA; Univ. of North Carolina, Chapel Hill, NC.

Background: ECG findings of QT-interval prolongation are associated with increased risk of coronary heart disease and of cardiac death. Although information on genetics of the congenital long QT syndrome is mounting, little is known about the genetics of QT interval in the general population. **Methods:** Heart rate adjusted QT interval (Bazett's QTc, and QT index (QTI)) was assessed by ECG in 2399 members (1270 female) aged 25-91 years in 468 randomly selected families participating in the NHLBI Family Heart Study. Familial correlation and segregation analyses were applied to evaluate whether familial/genetic factors and/or a major gene play a role in the variability of QTc and QTI in this population. **Results:** The parent-offspring (0.14±0.03) and sibling (0.18±0.03) correlations for age and sex-adjusted QTc were moderate and significant, while the spouse correlation was not different from (0.09±0.05). This suggests that there are familial/genetic influences on QT interval variability. Segregation analysis results suggest that there is a major effect in addition to heritable multifactorial effects ($h^2 = 0.34$). The major effect accounted for 11% of the QTc variation, but it did not follow Mendelian inheritance. Hence a major gene effect for QTc can not be supported. Further adjustments of QTc for serum cholesterol, blood pressure levels, current smoking, alcohol consumption, vigorous physical activity, BMI, and anti-arrhythmic medications did not significantly change the results of familial correlation or segregation analyses. Similar results were found for QTI. **Conclusion:** The QT-interval variation in the general population is influenced by moderate heritable multifactorial effects, while a major gene effect is not directly supported. Interestingly, these heritable effects appear to be independent of other major cardiovascular risk factors.

Program Nr: 443 from the 1999 ASHG Annual Meeting

Family-based association tests for markers on the X-chromosome despite of incomplete parental information. *S. Horvath[1,2], MP. Baur[1], NM. Laird[2], M. Knapp[1]*. 1) Inst. for Medical Statistics, University of Bonn, Bonn Germany; 2) Harvard School of Public Health Boston.

Family-based association methods have recently been introduced that allow testing for linkage and/or linkage disequilibrium between a marker and a disease even if there is only incomplete parental marker information (e.g. S-TDT by Spielman and Ewens 1998, Am J Hum Genet 62:450-458, SDT by Horvath and Laird 1998, Am J Hum Genet 63:1886-1897 and RC-TDT by Knapp 1999, Am J Hum Genet 64:861-870). All of these tests have been designed with autosomal markers in mind. Here we introduce the X-linked RC-TDT which like the (autosomal) RC-TDT is a non-parametric test that makes no assumption about the mode of inheritance of the disease and can thus be applied to complex X-linked diseases. Further it makes no assumption about the ascertainment of the sample and like most family-based association tests does not detect spurious association due to population stratification. The X-linked RC-TDT follows the same logic as the RC-TDT: First, one reconstructs the missing parental genotypes from offspring genotypes where possible. Second, similar to the TDT one counts the number of times a heterozygous mother transmits the interesting allele to the affected offspring. Third, one corrects for the bias introduced in the reconstruction step by conditioning on reconstructiblity. Even if only sons are affected (which could happen for an X-linked recessive disease) (unaffected) daughters are still very informative for reconstructing parental genotype information. We also discuss alternatives to the X-linked RC-TDT and apply the tests to real data.

Program Nr: 444 from the 1999 ASHG Annual Meeting

Breast cancer penetrance estimates for *BRCA1/2* from a segregation analysis of the pedigrees of mutation positive probands. *D.J. Kaufman[1], T.H. Beaty[2], J.E. Bailey-Wilson[3], J.P. Struewing[1]*. 1) Laboratory of Population Genetics, National Cancer Institute, Bethesda, MD; 2) Department of Epidemiology, Johns Hopkins School of Public Health, Baltimore, MD; 3) Inherited Disease Research Branch, NHGRI, NIH, Baltimore, MD.

Estimates of the risk of breast cancer for female carriers of germline *BRCA1/2* mutations have ranged from 27% to over 85% by age 70. A community-based study, the Washington Ashkenazi Study (WAS), collected personal and family histories of cancer and a blood sample on 5318 Jewish individuals. There were 114 unrelated *BRCA1/2* founder mutation carriers in this study. Using the "kin-cohort" method, the breast cancer penetrance was previously estimated to be 56% by age 70. There were 58 reported breast cancers among the 322 first-degree female relatives of the mutation carrier volunteers from WAS. Using data on these subjects, the REGTL module of the S.A.G.E. software modeled age-of-onset of breast cancer as the phenotype. Each of the 114 volunteer carriers was replaced in the data set by a woman affected at age 30, so the software would correctly identify her as a mutation carrier. Likelihoods were conditioned on the artificial carriers' phenotypes to avoid influencing the risk estimates. Estimated penetrance of breast cancer at age 70 is 61% under a dominant major gene model; across ages, penetrance estimates correspond closely to the age-specific risks calculated using the kin-cohort method. In non-carriers, segregation analysis predicted lower rates of breast cancer than did the kin-cohort method (6% versus 13% by age 70). This occurred because the estimated allele frequency was very high (0.22), so most cases were attributed to *BRCA1/2*. The model-based estimates of penetrance from the segregation analysis are very close to those obtained from the non-parametric kin-cohort method. A similar segregation analysis of ovarian cancer penetrance in *BRCA1/2* carriers is currently being performed.

Program Nr: 445 from the 1999 ASHG Annual Meeting

Single Nucleotide Polymorphisms: Mathematical modeling of the ascertainment bias and a comparison with sample distributions. *M. Kimmel*[1], *R. Chakraborty*[2], *L. Jin*[2], *R. Deka*[3]. 1) Dept Statistics, Rice University, Houston, TX; 2) Human Genetics Ctr, Univ Texas, Houston, TX; 3) Dept Environmental Health, Univ Cincinnati, Cincinnati, OH.

Single-nucleotide polymorphisms (SNPs) are considered an important new tool in the study of molecular evolution and gene mapping. One of the reasons is their frequent occurrence in the genome, estimated to be equal to 1 SNP per every kb of DNA. However, the SNP loci typed are subject to a serious ascertainment bias. The reason is that they are routinely discovered by reviewing sequences from a small number of individuals from a specific population. Therefore, more asymmetrically distributed polymorphisms will be frequently missed. We developed a mathematical model, based on a modification of the infinite sites model, which allows numerical predictions of the impact of ascertainment bias on the frequency distributions of SNPs, based on several scenarios of ascertainment. As an illustration, we provide a comparison with the distributions of nearly 400 SNP loci, each of which were originally screened in Caucasians, but studied now in six diverse populations. (Research supported by NIH grants GM 41399, GM 53545 and GM 45861, and by the Keck's Center for Computational Biology at Rice University).

Program Nr: 446 from the 1999 ASHG Annual Meeting

Effects of Stratification in the Analysis of Affected Sib-Pair Data: Benefits and Costs. *S.M. Leal, J. Ott.* Laboratory of Statistical Genetics, Rockefeller University, New York, NY.

The benefits and costs of stratifying affected sib-pair (ASP) data are examined in three situations where. 1.) There is no difference in identity-by-descent (IBD) allele sharing between stratified and unstratified ASP data sets. 2.) There is an increase in IBD allele sharing in one of the stratified groups. 3.) The data is stratified based upon IBD allele sharing status at one locus, and the stratified ASPs are then analyzed for linkage at a second locus.

Where there is no difference in IBD sharing between strata, a penalty is always paid for stratifying the data. The loss of power to detect linkage in the stratified ASP data sets is due to multiple testing and the smaller sample size within individual strata.

In the case where etiologic heterogeneity (i.e. severity of phenotype, age of onset, different ethnic backgrounds) represents genetic heterogeneity, the power to detect linkage can be increased by stratifying ASP data. This benefit is obtained where there is sufficient IBD allele sharing and sample sizes.

Once linkage has been established for a given locus, data can be stratified based upon IBD status at this locus, and tested at a second locus for linkage. In the case where the relative risk is in the vicinity of 1, the power to detect linkage at the second locus is always greater for the unstratified ASP data set. Even for values of the relative risk which sufficiently diverge from 1, with adequate sample sizes and IBD allele sharing, the benefits of stratifying ASP data are minimal.

Although stratification can be advantageous, it should be carried out with caution in order to avoid a potential loss in power to detect linkage.

Program Nr: 447 from the 1999 ASHG Annual Meeting

Haplotype-relative-risk-based evolutionary analysis of the tyrosine hydroxylase promoter region in manic depressive illness. *E.A. Lobos, R.D. Todd.* Dept Psychiatry, Washington Univ Sch Medicine, St Louis, MO.

Genetic association studies of the tyrosine hydroxylase (TH) gene and manic depressive illness (MD) have given conflicting results. Our earlier cladistics-based association analysis (CBAA) of TH haplotypes suggested that the locus was involved with MD susceptibility (Lobos and Todd, AJMG 74, 1997). CBAA has advantages over traditional association analysis because it models the evolution of the polymorphisms (PMs) at a genetic locus, but, when used in a case-control study, it does not control for genetic stratification. Thus, we re-tested the involvement of TH with MD using a sample of affected families. Haplotypes not transmitted to 72 affected offspring were used as a control sample to compare to transmitted haplotypes. A replication sample of 61 affected siblings was also analyzed. Seven PMs (including two from our prior report) were found within 600 bp of DNA sequence at the 5' end of TH. Nine haplotypes were found in our sample by PCR amplification and double digestion with restriction enzymes (ambiguous haplotypes were estimated). A cladogram (haplotype tree) was constructed to relate the nine haplotypes by 10 single-mutational steps. Our prior study of TH showed possible recombination within the region analyzed, resulting in an ambiguous cladogram. The smaller region studied here led to a simpler structure for analysis. A nested design for statistical testing was imposed on the cladogram. It created 5 one-step 'clades' (grouped closely-related haplotypes) and 2 two-step clades for frequency testing at higher levels of the hierarchy. We found no evidence for significant differences in frequency between the TH promoter haplotypes transmitted to affected offspring and those not transmitted. Testing higher-level clades also detected no significant frequency differences. Most studies using haplotype relative risk or transmission disequilibrium tests to explicitly control for genetic population stratification have ignored evolutionary information. In this study, we have combined CBAA with affected-family-based controls to make maximum use of haplotype information while controlling for both undetected population stratification and evolutionary process.

Program Nr: 448 from the 1999 ASHG Annual Meeting

Demographic, genealogical and genetic characterization of an isolated Sardinian micropopulation suitable for the study of complex traits. *P.M. Melis, L. Morelli, A. Angius, G. Casu, N. Ombra, S. Casula, G.B. Maestrale, D. Piras, S. Cabras, M. Pirastu.* Ist. Genetica Molecolare, C.N.R., Alghero, Sassari, Italy.

We have identified a very ancient (founded more than 1000 years ago) isolated village in a geographically isolated area of Sardinia to be used as an ideal model population for the identification of the genetic factors of complex disorders using an identity-by-descent (IBD) approach and linkage disequilibrium (LD) mapping. Blood samples were collected from 80% of the villagers. The original population size of about 200 has doubled between 1640 and 1870 and has reached 1200 inhabitants in 1990. A database was created using archival records (Quinque Libri) from the 17th century to 1990 in order to reconstruct the genealogy of the entire village. Village endogamy was about 95% with 35% consanguineous marriages for these centuries. Through the archives we reconstructed maternal and paternal lineages of all individuals and we complemented these data through Y chromosome haplotyping and mitochondrial DNA sequencing. We found that biological lineages were fewer than the one obtained through historical records: 75% of the actual population descends from 8 paternal and 10 maternal lines antedating the 17th century. In particular, 2 paternal and 4 maternal lineages include 50% of the population. We analyzed 50 unrelated males using six unevenly spaced genetic markers spanning a 12.5 Mb region on Chr Xq13. We identified 35 different haplotypes. Significant LD was observed between markers at 5 Mbp average distance. By overlapping the LD regions shared by different haplotypes we constructed LD contigs belonging to the village founders Chr X. We identified 5 ancestral X chromosomes shared by 80% of today inhabitants. We are studying some common diseases particularly frequent in the village (hypertension and nephrolithiasis). We correlated all the affected individuals and calculated all the meiotic steps separating them. This information in addition to the knowledge of the population structure are currently used to increase the efficiency of a multistep genome wide search for these complex disorders.

Program Nr: 449 from the 1999 ASHG Annual Meeting

Extensions of transmission/disequilibrium tests for correlated data or how to use your entire dataset for a test of linkage and association. *S.A. Monks*[1], *N.L. Kaplan*[2]. 1) Department of Biostatistics, University of Washington, Seattle, WA; 2) Department of Biostatistics, National Institute of Environmental Health Sciences, RTP, NC.

The transmission/disequilibrium test (TDT) on family data consisting of diseased child/parent trios has become widely used as a test of linkage and association. When genetic information from more than one diseased child per family is available, the TDT is no longer a valid test of linkage and association. Martin et al. (1997) noted that, by treating the transmissions from a heterozygous parent to their diseased children as a single unit, the TDT could be extended to utilize all diseased children while maintaining validity as a test of linkage and association. Much attention has been placed on extensions of the TDT for disease susceptibility loci, and extensions have also been proposed for quantitative trait loci. In particular, Rabinowitz (1997) proposed a method that is a valid test of linkage and association for data consisting of child/parent trios. Monks and Kaplan extended this test to allow for the use of data from additional children. Here, we demonstrate that each of these extensions can be obtained through the use of within cluster resampling (Hoffman 1999), a general method for analyzing correlated data. We then show that the TDT and Rabinowitz's method can be extended to tests of linkage and association for data consisting of pedigrees or a mixture of nuclear families and extended pedigrees, and that the resulting test statistic takes on a common and widely used form.

Program Nr: 450 from the 1999 ASHG Annual Meeting

Improved power of sib-pair linkage analysis using measures of complex trait dynamics. *J.H. Moore.* Program in Human Genetics, Vanderbilt University, Nashville, TN, USA.

There is a growing concern that methods of linkage analysis for complex traits have insufficient power to detect underlying loci. We investigated whether the power of sib-pair linkage analysis can be improved by measuring complex trait dynamics. We simulated complex traits using a deterministic nonlinear dynamic one-locus gene expression system with epistasis arising from feedback regulation and estimated the power of sib-pair linkage analysis for identifying the underlying QTL.

A total of 500 nuclear families consisting of two parents and two sibs were simulated for each of 1000 experiments. Markers with two alleles of equal frequency were simulated at 5 cM positions along one 100 cM chromosome. The marker at the 50 cM position was used as the QTL. The complex traits were simulated by iterating a deterministic nonlinear difference equation using initial values and parameter values drawn from genotype-specific normal distributions. The first trait was defined as the last value obtained from iterating the dynamic system 150 times and the second trait as the autocorrelation of adjacent values from the last 100 observations. The first trait represents sampling from a complex dynamic system at one point in time while the second trait represents measuring the dynamics from sampling at multiple time points. For each of the 1000 simulations we carried out a sib-pair linkage analysis of the two traits using the multipoint Haseman-Elston method and the nonparametric Wilcoxon rank-sum test approach. A single-test P-value £ 0.05 was considered statistically significant evidence for detecting linkage.

The power for identifying the QTL for the first trait was less than 10% while the power for identifying the QTL for the second trait was 100%. The difference in power reflects the difference between sampling a complex dynamic system at one time point and at multiple time points. It is anticipated that measuring traits from complex dynamic systems at multiple time points will improve the success of a proposed linkage study.

Program Nr: 451 from the 1999 ASHG Annual Meeting

Familial Aggregation of Stroke and its Relationship to Hypertension. *M.S. Nicolaou, A.L. DeStefano, I. Gavras, L.A. Cupples, A.J. Manolis, C.T. Baldwin, H. Gavras, L.A. Farrer.* Boston University School of Medicine, Boston, MA.

We evaluated patterns of familial aggregation of hypertension and stroke to test the hypothesis that inherited susceptibility to these disorders may be determined by a common set of factors. Genealogical and medical history information was obtained for a cohort of 354 hypertensive probands ascertained in a clinic-based setting, their 1427 first-degree relatives and 239 of their spouses. The risk of hypertension was higher for the parents and siblings of the probands than that for spouses (OR=2.4; 95% CI, 1.8-3.4; OR=2.2; 95% CI, 1.6-3.0 respectively). Using the spouses as a reference group the risk of stroke for parents of the hypertensive probands was 7.3 times higher (OR=7.3; 95% CI, 3.6-14.8) while a non-significant but slightly increased risk for siblings (OR=1.6; 95% CI, 0.8-3.3) was observed. Controlling for hypertension, obesity, smoking, coronary heart disease, diabetes and cholesterol resulted in decreased estimates of the risk of stroke for parents (OR=5.4; 95% CI, 2.6-11.2) and siblings (OR=1.2; 95% CI, 0.6-2.5). The risk of stroke was significantly higher for hypertensive parents and siblings than non-hypertensive parents (OR=5.2; 95% CI, 2.8-9.7) and siblings (OR=5.8; 95% CI, 2.1-15.9) while a history of hypertension was not associated with an increased risk for stroke in spouses (OR=0.7; 95% CI, 0.2-3.1). The risk of stroke in hypertensive relatives of probands with stroke was higher than that of the normotensive relatives (OR=13.4). A less elevated risk ratio was observed in the relatives of probands who did not have a stroke (OR=4.0). Our data showing a higher occurrence of hypertension and stroke in biological first-degree relatives of hypertensive probands than in spouses suggest that some of the genetic factors predisposing to these conditions may be the same. Elucidation of these factors through family studies of stroke may be difficult because of secular trends toward improved treatment for hypertension. Although a history of hypertension increases the risk of stroke among parents and sibs, multivariate analyses revealed a familial component to stroke independent of hypertension.

Program Nr: 452 from the 1999 ASHG Annual Meeting

An examination of association based tests for localizing genes. *D.M. Nielsen[1,2], B.S. Weir[1]*. 1) Program in Statistical Genetics, NC State Univ, Raleigh, NC; 2) Bioinformatics, Glaxo Wellcome, Research Triangle Park, NC.

It is generally accepted that the degree to which a disease susceptibility locus affects phenotype influences the power to detect and localize that locus. What is equally important is the manner in which these loci act. Since most association based measures examine alleles individually rather than as genotypes, it is reasonable to assume that tests based on these measures are effective at detecting genes with large additive effects. Loci which may have a substantial biological effect on the disease of interest, but which may not have a large additive component, might not be readily detected using these approaches. By applying insights from classical quantitative genetics, it is possible to formalize the relationships between markers, trait loci and phenotypes in a manner which allows us to determine which genetic properties association based tests have power to detect. We find the expected values of the statistics involved in these tests are sensitive only to the additive effects of the marker loci, while the variances are sensitive to both additive effects and dominance deviations. In a randomly mating population, the additive effects of a marker locus are a simple funtion of the additive effects at the trait locus and the linkage disequilibria between loci. Therefore, these factors have the largest effect on the power of an association based test to detect a trait locus. They are, however, confounded in the summary measures captured at the marker locus, and may cancel out when combined, so that large additive effects at the trait locus and strong disequilibria between loci are not sufficient to provide high power for detecting the trait locus.

In general, power to detect a trait locus using association based tests will depend on the way in which the trait locus affects phenotype and the manner in which the marker is associated with that trait locus. Because of this, some trait loci will not be readily detected by these methods. Additionally, care must be taken when comparing results across markers.

Program Nr: 453 from the 1999 ASHG Annual Meeting

HPCX (Xq27-28) linkage is common among Finnish prostate cancer families: Strong association with late onset disease. *J. Schleutker[1], M. Matikainen[2], A. Baffoe-Bonnie[1,5], J. Xu[3], P. Koivisto[2], T. Tammela[2], J. Smith[1], D. Stephan[1], W.B. Isaacs[4], J.M. Trent[1], J. Bailey-Wilson[1,5], O-P. Kallioniemi[1]*. 1) Cancer Genetics Branch, NHGRI, NIH, Bethesda, MD; 2) Laboratory of Cancer Genetics, IMT, TAUH, University of Tampere, Finland; 3) Center for Genetics of Asthma and Complex Diseases, University of Maryland, Baltimore, MD; 4) Department of Urology and Oncology, Johns Hopkins Medical Institutions, Baltimore, MD; 5) Inherited Disease Research Branch, NHGRI, NIH, Baltimore, MD.

In order to explore the genetic basis of prostate cancer susceptibility, we performed a nation-wide genetic epidemiological study in Finland, taking advantage of the genetically homogeneous population and cancer registry information. Fifty-seven informative families were studied by linkage analysis using microsatellite markers for the HPC1 region at 1q24-q25 (39 markers) and the HPCX region at Xq27-q28 (22 markers). Two point lod scores for all HPC1 markers were strongly negative, even in families with four or more affecteds and early age of onset. In contrast, lod scores for most markers at the HPCX region were mildly positive (max 2.05 at theta 0.14, marker DXS1205). Subgroup analyses suggested that most of the positive lod scores came from families with no male to male transmission (NMM) of disease (alpha 0.50 in this group), as could be expected for an X-linked disease. Maximum lod score in this group was 2.11 (theta 0.08, DXS1205). Sibpair analyses gave a lod score of 3.35 with DXS1205 ($p<0.00009$), implying that the generic model of disease transmission (Xu et al., 1998) used in the parametric analysis may be incorrect. Indeed, further stratified analyses indicated that virtually all late age of onset (average age at diagnosis >65 years) NMM families were linked (lod score 3.03 at theta 0, DXS1205), whereas those with early-age of onset were not. This suggests that HPCX linked families, which appear to be particularly common in Finland, may have a distinct phenotype, characterized by late age of onset.

Program Nr: 454 from the 1999 ASHG Annual Meeting

The Future of Genetic Case-Control Studies. *N.J. Schork[1,2,3,4], D. Fallin[1], X. Xu[3], M. Blumenfeld[2], D. Cohen[2]*. 1) Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH; 2) Genset Corp, La Jolla, CA & Paris, France; 3) Program for Population Genetics, Harvard School of Public Health, Boston, MA; 4) The Jackson Laboratory, Bar Harbor, ME.

The power and simplicity of the case/control study design makes it an appealing strategy for testing the relationship between particular genetic polymorphisms (or haplotypes) and traits or diseases. Despite this fact, there are a number of problems that plague the use and interpretation of genetic case/control studies. We describe methods that should allow researchers to evaluate and in many instances overcome many of these problems. These methods rely on the use of multiple genetic markers and can be used to investigate and control for the following problems: 1. genetic stratification; 2. allelic and locus heterogeneity; 3. indirect association and linkage disequilibrium; 4. sample heterogeneity; 5. the assessment of statistical significance; 6. the evaluation of the power and likely yield of a case/control study; and 7. The assessment and use of admixture. We showcase these methods with actual data and argue that because of its simplicity, the case/control design may become a design of choice rather than a design by default for future genetic epidemiology studies.

Program Nr: 455 from the 1999 ASHG Annual Meeting

Genetic epidemiology of Syndrome X disorders on Kosrae. *D. Shmulewitz[1], S.C. Heath[1], S.B. Auerbach[2], M.L. Blundell[1], J.D. Winick[1], S. Signorini[1], J.L. Breslow[1], J. Ott[1], T. Lehner[1], M. Stoffel[1], J.M. Friedman[1].* 1) Starr Center for Human Genetics, The Rockefeller University, New York, NY; 2) Health Resources and Services Administration, Department of Health and Human Services, New York, NY.

Syndrome X, a term used for the observed clustering of metabolic disorders, includes obesity, type II diabetes, hypertension, and dyslipidemia/heart disease. These disorders have been shown to have underlying complex genetic inheritance. We studied the prevalence and genetic epidemiology of these disorders on the Pacific island of Kosrae. This population based study included 2188 individuals (>90% of the adults on the island), of whom 1709 have been placed on a single pedigree. Participants in the study answered questions on their health status, family relationships, demographics and socioeconomic status. Subjects also underwent a clinical evaluation that included: anthropometric measures(weight, height, waist, hip), serum chemistries (leptin, fasting blood sugar [FBS], insulin, total cholesterol [TC], triglycerides [TG], apolipoproteins A1 [ApoA1] and B [ApoB]) and blood pressure (BP) measurements. Statistical analyses were carried out with the SAS package, and heritability and segregation analyses were performed using MORGAN and LOKI. The population on Kosrae showed, relative to the US, an increased prevalence of obesity (BMI>=35, 24%), diabetes (FBS>=126, 12%), hypertension (systolic BP>=140 or diastolic BP>=90, 17%), and dyslipidemia (TC>=240 or TG>=200 or ApoA1£88 or ApoB>=120, 20%). After adjusting for the covariates, sex, age, smoking, village of residence, and parity, heritabilities for the traits were calculated. They ranged from 0.20-0.64, indicating a significant genetic component for all. Segregation analyses showed significant evidence for multiple quantitative trait loci (QTL) for all the variables. Examples of major gene effects include BMI (25% of variance), FBS (51%), TC (41%), and BP (38%). This population is uniquely suited for further gene-mapping experiments, to find genes that are involved in the development of obesity, diabetes, hypertension, dyslipidemia, and their clustering in Syndrome X.

Program Nr: 456 from the 1999 ASHG Annual Meeting

Estimation of relative hazard for AIDS based on distributions of three HIV-1 resistant polymorphisms (SDF1-3'A, CCR2-641, CCR5-D32) in global populations. *G. Sun[1], B. Su[2], J. Xiao[3], F. Hu[3], D. Lu[3], R. Chakraborty[2], R. Deka[1], L. Jin[2,3]*. 1) Department of Environmental Health, University of Cincinnati, Cincinnati, OH; 2) Human Genetics Center, University of Texas Health Science Center, Houston, TX; 3) Institute of Genetics, Fudan University, Shanghai, China.

Chemokine receptors (CCR5, CXCR4 and CCR2) have been shown to be important coreceptors for HIV infection. Mutations at CCR5 (CCR5-D32), CCR2 (CCR2-641), and stromal-derived factor SDF1 (SDF1-3'A), a primary ligand for CXCR4, are known to have protective effects against HIV-1 infection. We studied the three-locus genotype frequency distributions in 70 worldwide populations from a sample of 2,341 individuals without any known history of the HIV-1 infection and AIDS symptoms. From these data, we estimated the AIDS onset risk (relative hazard, RH) of each population. The survey shows that the substantial frequency differences of each of these mutations translate into an extensive variation in RH for AIDS in worldwide populations. The estimated RH values are highly correlated (Spearman's r = 0.639, $P = 0.006$) with prevalence of AIDS. However, no evidence of natural selection against the mutant gene-carriers is detected. Finally, the combined three-locus genotype data predicts the highest RH in Southeast Asia and Africa, where AIDS is known to be more prevalent. (Supported by grants from the NIH and NSF).

Program Nr: 457 from the 1999 ASHG Annual Meeting

Evaluation of replication : The HLA-DR region in Rheumatoid Arthritis (RA) as an example. *F. Tores[1], M. Martinez[1], F. Cornélis[2], European Consortium on Rheumatoid Arthritis (ECRAF)[3].* 1) INSERM U358, Paris, France; 2) Université Paris 7-Denis Diderot; 3) EEC-BIOMED2.

Interpreting genome-wide scan linkage results has raised considerable debate. Guidelines have been proposed along with the use of genome-wide significance criteria for claiming significant or suggestive linkages. Linkage results must also be replicated. Replication studies do not face the multi-test problem of genome-wide screens. It has been shown, however, that initial positive results tend to overestimate genetic effects whereas subsequent studies will regress to the true value (Lander and Kruglyak, 1995; Suarez et al.,1994). Failure to replicate does not necessarily disprove the initial finding. However, it can also result from genetic heterogeneity. The replication dataset may not constitute a representative sample of the screen dataset, even though both samples are collected by the same investigators, using similar ascertainment and diagnostic criteria, as it is often done in a two-stage based genome-wide scan study. To investigate for genetic heterogeneity between two samples, we propose a bootstrap-based approach of the combined dataset. The goal is to quantify the probability of observing conflicting results when assuming both datasets are random samples of the same population. We illustrate this investigation in the ECRAF genome-wide scan data of RA. The HLA-DR region was tested within a 5cM region using three markers (D6S276, Tnfa, HLA-DR). The screen dataset (set1) is composed of 90 families (total of 105 ASPs). The replication dataset (set2) has similar sample size (96 families; 110 ASPs). Yet, significance for linkage, under pairwise or multipoint ASP analyses, is more than 10 times higher in set1 than in set2 (MLS=4.34 and 2.73, respectively). Simulations showed a poor empirical power of the linkage test power in set2. Bootstrap results did not reveal significant heterogeneity between the two datasets: variability between samples was not larger than that within samples, especially in set1. These results pointed out the sensibility of linkage test to the contribution of a small number of families, even in relatively large samples.

Program Nr: 458 from the 1999 ASHG Annual Meeting

Admixture Linkage Disequilibrium Mapping of Quantitative Trai Loci. *M. Xiong, L. Jin.* Human Gen Ctr/Houston HSC, Univ Texas, Houston, TX.

The linkage disequilibrium (LD) based methods are increasingly being used as tools for both initial localization and fine-scale mapping of human disease genes. However, the rapid attenuation of LD with the age of the mutation at the trait locus and the recombination fractions between the marker and trait locus makes it difficult to use LD-based methods in mapping genes involved in the complex traits in general populations. It has been recognized that the disequilibrium may result from recent admixture of populations in which the frequencies of the marker alleles and trait alleles are different. This principle has been sucessfully applied to the mapping of qualitative diseases, but less is known about its utility in mapping of quantitative trait loci (QTL). In this report, we propose a regression method based on LD for mapping QTL . Like TDT, the test statistic of this method is valid in the presence of population substructure. We investigated its power under three admixture population models: immediate population admixture model, general gene flow model, and island model and demonstrated that this method has high statistical power for any recently admixed population. The performance of this method is compared with transmission/disequilibrium test (TDT) designed for quantitative traits. The statistical power of the method for the selected sample using admixed populations is also evaluated.

Program Nr: 459 from the 1999 ASHG Annual Meeting

Direct observation of family history-taking in primary care practice. *L.S. Acheson, G.L. Wiesner, M. Goodwin, S.J. Zyzanski, K.C. Stange.* Case Western Reserve University, University Hospitals, Cleveland, OH.

Context: Primary care physicians are in a central position to identify families at risk for genetic abnormalities who may benefit from genetic consultation. Family history is the main clinical tool to screen for familial patterns of disease, but there are few data on family history-taking in primary care.

Objective: To describe the rate of family history-taking in community family practice and to identify characteristics of physicians, patients, and visits associated with obtaining family history information.

Design: Cross-sectional observational study in 84 community family practices in Northeast Ohio. Research nurses directly observed 4454 consecutive patient visits during 2 separate days in the offices of 138 family physicians.

Main outcome measures: Directly observed family-history-taking during an office visit and family history recorded in the office medical record.

Results: Family history was discussed during 51% of visits by new patients and 22% of visits by established patients. Younger physicians were more likely to obtain a family history; the rate varied from 0 to 81% of patient visits. Patients who were younger or middle-aged, married, female, and in better health were more likely to discuss family history during an office visit. Visits involving family history-taking were 3 minutes longer and more likely to be for well care rather than for acute or chronic medical problems. Discussions of family history averaged 2 minutes in length. Many patients who had no discussion of family history at the observed visit did have family history information in their medical records.

Conclusions: Family history-taking is highly variable from physician to physician. The amount of time used for family history-taking during any given visit to a family physician is small, but such information may be accumulated over multiple contacts, especially initial and well care visits. These findings may be used to design interventions to increase the rate and completeness of family history ascertainment.

Program Nr: 460 from the 1999 ASHG Annual Meeting

Cancer genetic clinics for hereditary breast/ovarian cancer (HBOC):differences between consultation content in Canada, France and United Kingdom. *L. Bouchard[1,5], F. Eisenger[2], G. Evans[3], W.D. Foulkes[4], B. Kerr[3], I. Blancquaert[1], H. Sobol[2], C. Julian-Reynier[5]*. 1) Universite du Quebec a Montreal and Council of Health Technology Ass., Montreal,Canada; 2) Inst.Paoli-Calmettes and E9901, Marseilles, France; 3) Dept.Medical Genetics, St-Mary Hospital, Manchester, UK; 4) Dept.Medicine, McGill University, Montreal, Canada; 5) INSERM U379, Marseilles, France.

The knowledge that BRCA1/BRCA2 are linked to HBOC plays a prominent role in clinical practice.Recommendations about clinical management seem influenced by cultural values.The objective of this study was to compare the content of cancer genetic consultations in 3countries.Method: Between 1996 and 1998,3cancer genetics clinics were surveyed:in Manchester(UK),Marseilles(F)and Montreal(Ca).Physicians completed a questionnaire on their patients'medical data and on their recommendations regarding prevention for 355 women attending the genetics clinic for the first time.Results:The women were 44 years old on average(16-76)and 33%had been diagnosed with cancer.In Marseilles(N=141)and Montreal(N=84), 78%of consultees were referred by a specialist compared to 32%in Manchester(N=130)($p<0.001$).Molecular analysis was offered more frequently in Marseilles(69%)and Montreal(59%)than in Manchester (48%)($p<0.005$).The topics discussed during consultation differed significantly according to the country.Environmental risk factors(diet, alcohol,tobacco)were more often recommended in Manchester(55%)than in Montreal(19%)and Marseilles(10%)($p<0.001$).In contrast,chemoprevention was mentioned more often in Montreal(42%)than in Manchester(24%)or Marseilles(14%)($p<0.001$).In Montreal and Marseilles, prophylactic mastectomy was discussed in 38% and 21% of cases respectively ($p=0.018$)and prophylactic oophorectomy in 44% and 14% of cases($p<0.001$).Manchester scored in between. These differences were more pronounced for women without cancer.Conclusion:These results seem to confirm the existence of a cultural dimension in clinical practice that might well be based on different traditions and beliefs regarding screening,treatment efficacy and the value of body integrity and fertility issues.

Program Nr: 461 from the 1999 ASHG Annual Meeting

Community-Based Recruitment Problems Encountered with a Colon Cancer Risk-Assessment Study. *M. Cappelli[1], H. Stern[3, 4], K. O'Rourke[3, 4], S. Viertelhausen[3, 4], L. Van Houten[1], A. Hunter[2]*. 1) Psychology, Children's Hospital of Eastern Ontario; 2) Genetics, Children's Hospital of Eastern Ontario; 3) Surgery,University of Ottawa; 4) Loeb Research Institute.

It has been postulated that Ashkenazi Jews have a higher risk of colorectal cancer than the general population. A study assessing the risk of colon cancer in the Ashkenazi Jewish population in the Ottawa-Carleton region was conducted recently. A community-based recruitment approach was used to target close to all the adult Ashkenazi Jews in the community. Of the 3548 households in which packages were mailed to, only 1595 responded, leaving 1953 households who did not respond. The main objectives of the present study are to assess the effectiveness of using a community-based approach for recruiting at-risk families and to examine the factors that determined participation and response rates in the risk-assessment study. Six hundred individuals from three different groups are currently being recruited to participate in a standardized telephone interview. The three groups include: those who particiapted in the risk-assessment study, those who responded and refused, and those who did not respond. We anticipate that factors related to individuals' attitudes and perceptions of colon cancer, physicians' input, past health prevention behaviour, recruitment methods, and personal and family history of cancer will be associated with participation and response rates in the risk assessment study. We expect to find that those with increased knowledge about colon cancer and its prevention, those whose physicians have recommended colon screening and those who practice greater health prevention behaviours will have been more likely to participate in the colon cancer risk assessment study. We also expect to find that the advertisements at synagogues and the Jewish Community Centre will have been less effective methods of recruitment than perhaps the mail-out, which is a more active form of recruitment. From these finfings we hope to develop effective strategies which may improve participation and response rates in similar future studies geared towards disease prevention.

Program Nr: 462 from the 1999 ASHG Annual Meeting

Dissent as a Mechanism to Provide Choice in Population-Based Public Health Studies: Implementation and Preliminary Analysis. *A.M. Comeau[1], R.B. Parad[1,2], R. Eaton[1].* 1) New England Newborn Screening Program, U Mass Medical School, Boston, MA; 2) Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

What are the criteria by which disorders are added to the list of mandatory newborn screens? And given those criteria, which, if any disorders should be added to the list in Massachusetts? Advances in technology, recognition that early detection and intervention involve a spectrum of outcomes, and evolving definitions of "mandatory" and "pilot" programs in keeping with ethical, legal and social mores point to the need for a systematic review of options. After an independent advisory board affirmed the rationale for the 9 mandated screens in MA, it added a 10th (MCAD) and recommended that screening for 19 other metabolic disorders detectable by MS/MS and screening for cystic fibrosis be offered to the MA birthing population in the form of two pilot studies. Human subjects review required informed consent for the pilot studies, and approved an alternate form of informed consent, verbal 'consent or dissent'. Implementation included development of written materials for parents and providers, in-service and grand rounds trainings of providers, distribution of materials and QA/QC monitoring. As of April 30, parents of 17,992 newborns have been offered the opportunity to participate in either or both of the studies. A total of 401 (2.2%) declined participation in either study, with smaller numbers declining one or the other study. A substantial overall increase in awareness and understanding of newborn screening is reported. Other analyses will be presented.

Program Nr: 463 from the 1999 ASHG Annual Meeting

Genetic testing for Cystic Fibrosis : Evaluation of three European Quality Assessment trials. *E.M.C. Dequeker, J.J. Cassiman.* Center for Human Genetics, KULeuven, Leuven, Brabant, Belgium.

Within the framework of the European concerted action on cystic fibrosis (CF) yearly quality assessment trials have been set up since 1996 in order to evaluate the quality of the genetic testing for CF performed in diagnostic laboratories. The large majority of European laboratories providing genetic services for CF participated (1996: n=136, 1997: n=145, 1998: n=159). Successive quality control trials resulted in a gradual reduction of the percentage of laboratories making one or more mistakes on a total of 12 alleles to be examined (1996: 35%, 1997: 24%, 1998: 21%), although the error rate remains unacceptably high. The types of error made ranged from administrative errors, erroneous technical results to misinterpretation of the (technical correct) data. The annually organised QA schemes also provide a nice tool to evaluate the evolution in testing strategies. There is a tendency in molecular diagnostic laboratories over the past few years to shift to the use of commercially available kits. Almost 50% of the participants used a commercially available kit as primary testing tool for screening of DNA samples for mutations in the CFTR gene. The results of the QA scheme of 1998, however, showed that the use of a commercial kit alone does not necessarily ensures a high accuracy of mutation analysis. We conclude that external control trials contribute to an overall improvement of the quality of genetic testing. However, continued efforts will be needed to further improve the genetic services provided to the community.

Program Nr: 464 from the 1999 ASHG Annual Meeting

Consumer interest in genetic testing for breast cancer susceptibility: Variation with test and gene characteristics.

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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 mailed to: 1) adult female KP Northwest health plan members unselected for personal or family cancer history; 2) women with only a family history of breast cancer; and 3) women with breast cancer and a family history of breast cancer. Respondents were predominantly white non-Hispanic (77%; 10% black; 2% Asian/Pacific Islander; 1% white Hispanic; 1% Native American/Alaskan; 8% mixed race/other); median age was 59 years. 21% appeared to have difficulty interpreting numerical risks.

Results indicate that interest in genetic testing increases with a higher prior probability of carrying a deleterious mutation and with higher penetrance (breast cancer risk associated with a mutation), but does not vary with positive or negative predictive values of a genetic test. 31% would definitely want testing if they had a 1 in 10 prior probability, 19% for 1 in 100, and 14% for 1 in 1,000. 55% would definitely want testing if penetrance were 95%, 49% if it were 75%, 36% if it were 50%, and 24% if it were 25%. This pattern of results was similar for the three mailing groups described above, as well as by age, numeracy, and previous information about breast cancer and genetics. In conclusion, gene characteristics may affect individuals' decisions about genetic testing. Consumer preferences should be considered by policymakers when recommending strategies for genetic testing.

Supported by NCI P01 CA72085 and PHS T32 HS00069.

Program Nr: 465 from the 1999 ASHG Annual Meeting

Ten Years of Minimum Data Collection - New York State's/GENES' Experience. *K.B. Harris, K.A. Pass.*
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In 1987, the NYS Genetic Services Program began collecting individual patient contact forms from clinical genetic service units in response to the development of the CORN Minimum Data Set. All funded providers were and are required to submit information on each patient receiving face-to-face genetic services. Many programs not receiving funding also provide information. Data collected include a unique patient identifier, service date, provider zip code and county of residence, age, sex, race, ethnicity, payer (insurer), reason for visit, cognitive and clinical services provided, diagnostic tests ordered and diagnosis.

Uniform data collection of services as difficult to define as those provided by clinical genetic programs is problematic, as demonstrated by criticisms of the CORN Minimum Data Sets of 1987-1994. Some of the limitations of the NYS/GENES database will be discussed, including full reporting, clerical mistakes, validation of data, and maintaining committment of the non-funded providers.

Given those caveats, however, the data set, which includes individual patient information on 350,000 visits by 285,000 patients at 47 clinical genetics service units in NYS offers valuable insight into the evolution of public health genetics, clinical genetic services and the medical and lay communities' perception of the worth of these services. Maps of NYS and New York City demonstrate access to services geographically; graphs show trends of access by those of different ethnicities and financial status. Programmatic knowledge of federal SPRANS priorities, availbility of health insurance, state funding levels for genetics services and institutional commitment to genetic services illustrate the impact of policy changes on accessibility of genetics to populations throughout NYS.

Program Nr: 466 from the 1999 ASHG Annual Meeting

Identification of family members at risk for hereditary breast and ovarian cancer and up-take rates for predicitive testing: A report from a 3 year multi-disciplinary clinical study. *I. Nippert[1], B. Dworniczak[1], B. Ziese[1], C. Jackisch[2], S. Preisler-Adams[1], J. Horst[1]*. 1) Department of Human Genetics, Westfaelische Wilhelms-University (WWU), Muenster, Germany; 2) Women`s Hospital, WWU, Muenster, Germany.

Introduction: In January 1997 a multidisciplinary BRCA1/BRCA2 predictive testing program was implemented at the Westfaelische Wilhelms-University Muenster, Medical School. The test is offered - free of charge - to individuals and families who meet defined high risk criteria. Individuals and families follow a thorough pre- and post-test counseling protocol in order to ensure informed consent and client autonomy. **Methods:** In order to assess uptake rates of individuals at risk for hereditary breast and ovarian cancer their decision making process is recorded. **Results:** Since January 1997 to May 31th, 1999, 314 families have been seen for pre-test counseling. Of these families 206 (65.6%) met the inclusion criteria and were offered the test. 52 (25.0%) declined the offer, 23 (11.2%) could not be tested because the index patient refused to be tested or could not be reached, 22 (10.7%) are still considering the offer and 109 (52.2%) took up the test. So far a mutation has been found in 14 (12.8%) families, none was found in 23 (21.1%) and in 72 the test is still running. In the 14 affected families, 61 first grade relatives at risk were identified. It is up to the counsellees to inform their relatives about the risk. 9 relatives (14.7%) took up the test, 5 (8.2%) declined the offer, 4 (6.6%) are still undecided and of 43 (66.1%) it is unknown whether or not they have been informed by their affected relative. Of the 9 persons tested, 6 had a positive test result, of these 6 persons 4 chose to be informed, 1 decided not to know the test result and 1 person's decision is still pending. **Conclusion:** In genetics the true patient is a family with a shared genetic heritage. But in modern society families seem to be fragmented rather than unified and in most families identified to carry a mutation, affected members seem to be willing to disclose the findings only to a chosen set of relatives.

Program Nr: 467 from the 1999 ASHG Annual Meeting

Genetic testing in children: the "Rule of Earliest Onset" falls short of the mark. *M.Z. Pelias.* Dept Biometry & Genetics, Louisiana State Univ Med Ctr, New Orleans, LA.

Questions about genetic testing in children have been the subject of concern for almost one decade. Professionals in cancer genetics have recently suggested that minors be tested according to the "Rule of Earliest Onset," which mandates testing minors no earlier than the age of first possible onset of disease. Justifications for this rule include potential stigmatization and discrimination, maximizing the minor's participation in deciding to proceed with testing, and allowing medical benefits to accrue to the child. While this approach is logical for early onset cancers, it fails to acknowledge other interests of parents and other benefits that their children could enjoy. Interests of parents derive from their right and duty to make decisions for their minor children. Genetics professionals who presume that parents will not make decisions that are in the "best interests of the child" usurp the parental prerogative and improperly insert themselves into the privacy of family and parents. Rather, professionals should provide thorough counseling, with a presumption of deference to parents who are trying to do their best for their families. Among benefits for children is the primary concern for their health. In addition to medical benefits, however, are numerous other benefits for children, including resolution of uncertainty and anxiety, increased communication and candor within the family, opportunities for emotional and financial adjustment to the family's genetic situation, understanding and compassionate assessment of life prospects, and even acceleration of the maturation of the child toward his or her own nascent autonomy. While these and other benefits can be viewed from the perspective of their concomitant risks, the risks are perhaps best appreciated when they are acknowledged and thoroughly considered during the counseling process prior to testing. Parents who are struggling to do their best for their families need all the help they can get, and they do not need artificial or arbitrary obstacles to thwart their efforts.

Program Nr: 468 from the 1999 ASHG Annual Meeting

Services for the prevention and management of genetic disorders and birth defects in developing countries. *V.B. Penchaszadeh[1], A. Christianson[2], R. Giugliani[3]*. 1) Div Medical Genetics, Beth Israel Medical Center, New York, NY, USA; 2) Dept Human Genetics, University of Pretoria, Pretoria, South Africa; 3) Dept Medical Genetics, University Hospital,UFRGS, Porto Alegre, Brazil.

While genetic services have had an explosive development in the industrialized world, they are lagging in developing countries. An Advisory Group constituted mostly by geneticists from 13 developing countries was convened on January 5-7, 1999 by the World Health Organization and the World Alliance of Organizations for the Prevention of Birth Defects, to assess and make recommendations on genetic services in the developing world. As rapporteurs of the meeting, we present its main conclusions and recommendations. Genetic diseases and birth defects occur in developing countries at equal or higher frequencies than in the industralized world and their impact on health and quality of life is greater because of deficient services and the load of infections and malnutrition. The role of genetic factors in disease, however, is not sufficiently aknowledged. Recommendations of the Group included: need that health professionals and public health officials recognize the burden imposed by birth defects and genetic disorders; need for political will and commitment for their prevention and management; improve knowledge on the epidemiology of birth defects and genetic disorders; define goals of genetic services in terms of individual and family wellbeing as well as of public health; improve reproductive health, prenatal and newborn care, including attention to maternal age, nutrition, vitamin supplementation and avoidance of teratogens; organize genetic services in a comprehensive manner, integrated with other relevant health services, rooted in the primary care level, with proper referral channels to existing genetic centers; prioritize prevention programs and service targets according to prevalence, severity and the predicted outcomes of measures; respect ethical principles and cultural diversity; train health professionals in genetics; educate the public in genetics; encourage the formation of and support parent/patient organizations. Advocacy for these recommendations is taking place internationally.

Program Nr: 469 from the 1999 ASHG Annual Meeting

How do U.S. Laboratories do Huntington Disease testing for at-risk individuals? *W.K. Seltzer[1], N. McIntosh[2], R.H. Myers[3], The US Huntington's Disease Genetic Testing Group[1,2,3]*. 1) Athena Diagnostics, Worcester, MA; 2) DIANON Systems, Inc., Stratford, CT; 3) Massachusetts General Hospital, Boston, MA.

The clinical, psychosocial and ethical implications of molecular diagnostic testing for Huntington disease (HD) in at-risk individuals are profound. Practice guidelines were established in 1994 by the American Academy of Neurology and the Huntington Disease Society of America (Neurology 1994; 44:1533-1536) to aid clinicians and laboratories in providing maximum benefits to their patients while minimizing the potential risks.

A survey was faxed to 33 US labs listed as offering HD testing in the Genetests™ directory. The survey was devised to assess (1) information that is minimally required by labs to process a specimen for at-risk HD testing; (2) how closely labs are adhering to the guidelines; and (3) current attitudes towards the lab's role as 'gatekeeper' in assuring compliance with the guidelines. Nineteen labs (60%) returned completed surveys.

Most labs reviewed requisitions (18/19), noted the age of the patient (17/19) and documented receipt of consent forms (16/19), prior to processing a patient's specimen. Information most often required included a patient identifier (13/19) and date of birth (15/19); declaration of the patient's clinical status (15/19); documentation of counseling (14/19), and testing being requested by a medical professional (15/19).

Most labs (15/19) stated that they generally followed the guidelines, but exceptions had been made in unusual cases. When asked if a lab would be in favor of shifting the responsibility of complying with the guidelines to the requesting physician, no consensus was reached.

Results from this survey suggest that labs are requiring or requesting much the same information before processing at-risk HD specimens, and are generally adhering to the guidelines. Labs, however, are divided when it comes to relinquishing the responsibility of 'gatekeeper' to comply with them.

Program Nr: 470 from the 1999 ASHG Annual Meeting

Genetic counseling for retinoblastoma (RB) in the new millenium: The implications of identifying RB gene mutations. *J. Sutherland[1], R. Panton[1], J. Anderson[1], L. Han[1], B. Gallie[1,2].* 1) Eye Gen Team, Ophthalmology, Hosp Sick Children, University of Toronto, Toronto, ON, Canada; 2) Ontario Cancer Institute/Princess Margaret Hospital, University Health Network, Univeristy of Toronto, Toronto, Canada.

The RB gene mutation has been identified and reported by our laboratory for 225 probands and 290 of their relatives. Of the 170 isolated cases, 104 were bilaterally affected. All their tested parents were normal except for two families, suggesting that the mutations had arisen *de novo* in the majority of probands. Of the 66 unilateral isolated cases, 60 were normal for the two RB gene mutations found in the proband's tumor, implying that the chance of tumors developing in the unaffected eye and other lifetime cancer risks are reduced. Thus for 164 families, the risk for others to be affected is close to the population risk, while a <1% risk of mosacism remains. Siblings and cousins in these families are spared invasive clinical screening. The cost of molecular screening found equal to conventional screening for the immediate generation, and 5-fold less when future offspring of 64 probands were considered. Despite having no family history, 6/60 probands with unilateral tumors had germline RB gene mutations, indicating that their relatives required screening, just as familial RB relatives. We utilize a prenatal decision tree for genetic counseling discussion of options. When molecular tests indicate that the infant has the family's RB gene mutation, imposing a >90% risk to develop RB tumors, we include the option for early delivery at 36 weeks, in order to maximize visual outcome and chance for cure, and minimize treatment morbidity.

Program Nr: 471 from the 1999 ASHG Annual Meeting

Changing economic patterns in prenatal cytogenetic diagnosis. *P. Wyatt, A. Summers, A. Neidhardt, S. Olsen, E. Mak-Tam.* Dept Genetics, North York General Hospital, Toronto, ON, Canada.

Historically, the prenatal diagnosis of fetal chromosomal disease has been based on using advanced maternal age as the initial screening tool. This resulted in large volume testing of specimens with low abnormality rates. Many of the fees negotiated to pay for these cytogenetic services have been based on processing high volumes of low risk specimens. Changing pregnancy screening techniques have replaced maternal age as the primary screening tool with methods such as maternal serum screening (1st and/or 2nd trimester testing), second trimester ultrasound changes, and, recently, even first trimester ultrasound changes such as nuchal tranlucency, have been added to the techniques used to evaluate the risks of chromosomal disease in pregnancy. This will likely lead to a substantial change in the proportion of cases with abnormalities in cytogenetic laboratories. This change in abnormality rate and complexity per case processed in each laboratory may necessitate an adjustment of the fees paid for cytogenetic analysis by governments/health care organizations. Analysis of the information available from one of Canada's largest genetic centers confirms that the abnormality rate in the cytogenetic laboratory has changed from a rate of 1 autosomal aneuploid per 120 specimens in 1983 to a rate of 1 autosomal aneuploid for every 40 specimens by 1998. Initial information confirms that the use of new markers will continue to increase the abnormality rate in 1999 - anticipated to reach a rate of 1 abnormal per 20 specimens received. Objective data demonstrating the changing complexity of genetic care has remained difficult to quantitate. The area of changing complexity of prenatal cytogenetic service confirms the rapid change that is occurring in genetic care and may be of assistance to quantify the change to the various agencies involved with payment.

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Program Nr: 472 from the 1999 ASHG Annual Meeting

On the use of population-based registries in the clinical validation of genetic testing for disease susceptibilities.

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Many new genetic tests for susceptibility to adult-onset diseases are developed on the basis of selected and high-risk groups. Before such tests can be used in medical practice, however, epidemiologic studies must be conducted to evaluate their clinical sensitivity, specificity, and positive predictive value in the general population. For many common adult-onset diseases, this process may take decades of follow-up. Here, we illustrate how clinical validation of new predictive genetic tests can be done retrospectively using case-control studies that are derived from population-based registries of diseases. We use the examples of birth defects and cancer registries to illustrate a hypothetical process by which such tests can be clinically validated. We demonstrate how such epidemiologic studies can be successfully used to derive measures of a test's sensitivity, specificity, positive predictive value, negative predictive value and of the population attributable fraction of disease due to the disease-susceptibility genes. Under certain assumptions, data derived from population-based case-control studies provide adequate estimates of lifetime risks for disease (penetrance) among people with specified genotypes. With adequate protections of human subjects, studies involving population-based registries of disease will increasingly become a valuable asset in effort to validate the numerous genetic tests that will emerge from advances in human genetic research and the Human Genome Project.

Program Nr: 473 from the 1999 ASHG Annual Meeting

Simulation of gene expression patterns in cDNA microarray data. *L.R. Bailey, J.H. Moore.* Program in Human Genetics, Vanderbilt University, Nashville, TN.

The development of cDNA microarray technology has facilitated the simultaneous measurement of the relative expression levels of thousands of genes. The goal of this study was to develop a computational methodology for simulating gene expression patterns in cDNA microarray data. We required this method to be simple enough to be implemented with any statistical or spreadsheet software package and to be flexible enough to generate patterns for any number of gene expression variables and with any defined mathematical relationship among them.

To accomplish this goal we developed a simulation methodology that consists of the following five steps. First, generate random deviates from a particular statistical distribution for the number of variables to be modeled and for the total number of observations present in all biological or clinical endpoint groups. Second, define any parameters to be used and the mathematical relationship between the gene expression variables and the parameters. Third, apply the mathematical function to the parameters and variables to generate a new variable. This new quantitative dependent variable will be used to generate a dichotomous or polytomous dependent variable representing the functional biological or clinical endpoint groups. This is accomplished in step four by sorting the quantitative dependent variable in ascending or descending order and assigning group values to sequential blocks. The fifth and final step is to place the independent variables in the same observational order as the quantitative dependent variable. This final step generates a pattern specified by the mathematical function that is associated with the new dichotomous or polytomous dependent variable.

We illustrate this methodology by simulating different patterns of gene expression among multiple genes and assessing the power of linear discriminant analysis and a feed-forward neural network for detecting these simulated patterns. We expect cDNA microarray datasets simulated in this manner will be useful for developing and testing new pattern recognition methods and will be useful for assessing the power of a proposed microarray study.

Program Nr: 474 from the 1999 ASHG Annual Meeting

Comparative analysis of an evolutionary chromosomal breakpoint indicates a recent origin for the human 4q telomere. *D.J. Bolland[1,2], M. van Geel[3], L. Carim Todd[2], R.R. Frants[4], P.J. de Jong[3], J.E. Hewitt[1,2]*. 1) Institute of Genetics, University of Nottingham, Nottingham, UK; 2) School of Biological Sciences, University of Manchester, Manchester, UK; 3) Department of Cancer Genetics, Roswell Park Cancer Institute, Buffalo, NY; 4) MGC-Department of Human Genetics, Leiden University Medical Centre, Leiden, The Netherlands.

Comparative mapping and sequence analysis may be used to identify conserved sequences and to investigate chromosome evolution. We have been focusing on the subtelomeric region of human chromosome 4q, the candidate region for facioscapulohumeral muscular dystrophy (FSHD). The mechanism most likely to be causing FSHD is a position effect. FRG1, the most telomeric gene on 4q, was used as the starting point for development of comparative gene maps of this region. The Japanese Pufferfish, Fugu rubripes, has a similar gene complement to human in a genome estimated to be only 400Mb, making Fugu an ideal organism for cloning human disease genes. We sequenced a 40kb cosmid containing the Fugu homologue of FRG1. Adjacent to FRG1 in this cosmid are the homologues of two human chromosome 8p22 genes, acid ceramidase (AC) and pericentriolar material gene 1 (PCM1). We have shown that the mouse homologues of FRG1, AC and PCM1 are also tightly linked and map to the central region of chromosome 8. Sequencing of a 180kb mouse PAC (RPCI21-44K14) revealed both gene order and orientation to be the same in mouse and Fugu. In addition to the Fugu and mouse clones, we have sequenced a 130kb human 4q35 PAC containing FRG1 (RPCI1-226K22). The availability of 350kb of sequence containing the human AC and PCM1 genes from chromosome 8p22 will enable a detailed comparative study of the breakpoint region. Using BACs, we have demonstrated that the FRG1-AC-PCM1 gene linkage group is also conserved in pig, cow and dog but not in chimpanzee and baboon, suggesting that the disruption of this linkage group arose relatively recently in the primate lineage. These data suggest recent telomere formation in the forerunner of human chromosome 4q and fit the proposed position effect hypothesis whereby the FSHD gene was moved to a location unfavourable for normal expression.

Program Nr: 475 from the 1999 ASHG Annual Meeting

Gene isolation on human chromosome 7q: Identification of the type II citrullinemia gene and a splenic lymphoma gene. *A. Boright[1], M. Corcoran[2], D. Oscier[2], S. Mould[2], K. Kobayashi[3], T. Saheki[3], D. Sinasac[1], J. Rommens[1], L.C. Tsui[1], S. Scherer[1]*. 1) Dept Genetics, Hosp Sick Children, Toronto, ON, Canada; 2) Royal Bournemouth Hospital, UK; 3) Dept. of Biochemistry, Kagoshima University,.

As part of our effort to generate a gene map of human chromosome 7 we are analyzing genomic DNA sequence using a compendium of bioinformatic tools followed by wet-bench experimentation for full-length transcript identification. Initially, we focussed on the 7q21-q22 region since it contains the loci for many diseases including, NIDDM, myeloid leukemias and lymphoproliferative disorders. We have analyzed exhaustively a 15 Mb region for which complete genomic sequence exists and have been able to identify over 95 genes that could be annotated based on known or inferred function. Our analysis indicates the average gene is 54 kb in length, contains 12 exons and encodes a mRNA of 2.5kb. For some regions the GC content exceeds 60-70% representing H3 isochores. We have integrated into a single map for 7q21-q22, all of the DNA markers and genomic clones for disease gene studies. Using this information we have recently identified the gene that causes type II citrullinemia (Nature Genetics 22, 159-163, 1999) and another (CDK6) involved in splenic lymphoma. For the latter, the analysis of chromosomal translocations in four patients identified the site of rearrangement upstream of the CDK6 cell cycle gene leading to a marked overexpression of CDK6 . In 3 of the patients with a t(2;7)(p11;q22) karyotype, DNA sequencing of the breakpoint revealed it was located 3.8 kb 5'-of exon 1 and involved aberrant VJ recombination between the immunoglobulin light chain region on chromosome 2p and DNA sequence at 7q21 resembling the heptamer recombination signal. In a fourth patient, the breakpoint resided 66 kb upstream to exon 1 juxtaposing CDK6 to an uncharacterized transcript. The analysis of the genomic sequence surrounding CDK6 identified additional potential heptamer recombination signals and these sites are being characterized in other clinical samples with 7q21 rearrangements.

Program Nr: 476 from the 1999 ASHG Annual Meeting

SNP determination using cleavable primers and time-of-flight mass spectrometry. *D.C. Chen, S. Royer, S. Nieto, H. Lin, J.M. Butler, J.A. Monforte.* Biotechnology, GeneTrace Systems Inc., Alameda, CA.

Single nucleotide polymorphisms (SNPs) are the most frequent form of DNA sequence variation in the human genome and as such are becoming increasing popular genetic markers for genome mapping studies, medical diagnostics, and identity testing. The recent acceleration of sequencing efforts in the Human Genome Project is expected to result in more than 100,000 human SNPs for analysis within the next two or three years. Automated, high volume testing procedures will be required to score multiple samples and examine population variation across these SNP markers. To complement its target discovery process, GeneTrace Systems has developed robotic sample processing, automated time-of-flight mass spectrometry, and rapid genotyping software that is capable of analyzing thousands of samples daily. We use a primer extension assay with a novel cleavable primer that improves the capability for accurately calling heterozygote samples and extends the range for non-overlapping multiplexes. Multiplex SNP results will be illustrated from a number of marker systems including mitochondrial DNA and Y chromosome polymorphisms. In addition, SNP data from a subset of the 450 samples in the NIH DNA Polymorphism Discovery Resource will be shown across several human SNP markers.

Program Nr: 477 from the 1999 ASHG Annual Meeting

STACK_PACK and STACK (Sequence Tag Alignment and Consensus Knowledgebase): A novel, comprehensive, hierarchical EST clustering and consensus generation and analysis system providing unique insight into the human genome. *A. Christoffels, R. Miller, W. Hide.* SANBI, University Western Cape, Bellville, Wp, South Africa.

The sequence errors characteristic of ESTs together with their fragmented nature makes them unsuitable for accurate assessment of gene biology and expression. We hierarchically organise and incorporate as much EST information as possible resulting in long consensus sequences, gene expression variants, sequence quality and templates for protein translation. The STACK_PACK system employed does not rely on specific clustering algorithms, but rather approaches the problem of management viewing integration and analysis of the clustering data. We have successfully employed STACK_PACK to generate a highly processed, hierarchically organised database, STACK (http://www.sanbi.ac.za/stack/).We have performed tissue and index level organisation and have processed GenBank release 110 (151098) to reduce the ~1.3 million input ESTs to 173740 STACK sequences. These sequences are represented in 16 tissue divisions. We have also generated a 'high confidence' SANIGENE dataset comprising 95814 sequences where each consensus sequence is represented by at least 2 sequence reads. In order to improve the information content of each cluster, clusters that share clone-IDs were linked and resulted in 76155 linked sequences with an average of 1400.6 bases.. In addition to the tissue divisions, we have generated a whole body index by clustering 330000 STACK tissue consensi on a 128 CPU SGI-CRAY Origin 2000. The index comprises 141291 STACK-INDEX sequences (67000 3' unique sequences) and 23553 SANIGENE-INDEX sequences. With the addition of the index, our database can be browsed hierarchically from the EST level, through the tissue state to the index level using a Perl-object html system (http://ziggy.sanbi.ac.za/cgi-bin/showCLUSTER.pl) that clearly shows the assembly paradigm, and delivers information on alternate expression forms of genes. We are now employing the system to develop a dataset of alternately expressed cancer forms of genes.

Program Nr: 478 from the 1999 ASHG Annual Meeting

Fine mapping of an X-breakpoint from a balanced (X;3) translocation in a female patient with nonspecific mental retardation (MRX), and identification of a new MRX candidate gene. *L. Crisponi[1], M. Uda[1], M. Rocchi[2], P. Ma[3], E. Chen[3], S. MacMillan[4], D. Schlessinger[5], L. Boccone[1], A. Cao[1], G. Pilia[1].* 1) IRTAM, CNR, Cagliari, Italy; 2) Univ. di Bari, Bari, Italy; 3) Perkin-Elmer Co., Foster City, CA; 4) Washington University, S. Louis, MO; 5) National Institute of Aging, HIH, Bethesda, MD.

X-linked Mental Retardation (XLMR) can be roughly categorized as syndromic (MRXS) or nonspecific (MRX). Linkage studies in single large MRX families have identified approximately 60 loci that fall in 8-10 non-overlapping regions along the X chromosome, suggesting the involvement of at least 8-10 corresponding X-linked genes. So far four genes responsible for MRX have been cloned (FMR2, oligophrenin-1, GDI1, PAK3). For the remaining genes, linkage intervals are still too wide to permit identification of causative gene defects by positional cloning. We have studied a female patient with a balanced de novo translocation t(X;3)(q13.1;p14) associated with mental retardation. A number of MRX loci(MRX 4,5,8,13,14,20,22,26,31, 52 and 58) have been mapped to the Xq13 region, so that the X breakpoint in this patient might be associated with one of them. Yeast Artificial Chromosome (YAC) and Bacterial Artificial Chromosome (BAC) clones were used as probes in fluorescent in situ hybridization (FISH) analysis to map the breakpoint to a 150 kb interval. A 150 kb BAC spanning the breakpoint has been completely sequenced, and using Southern blotting experiments with genomic probes designed to identify all the restriction fragment of the critical region, we have been able to map the breakpoint within a 3 kb interval. GenBank comparisons found several Expressed Sequence Tags(ESTs) in the vicinity, and computational searches with GRAIL2 predicted several additional potential exons. A cDNA cognate for sequence from one of the potential exons was recovered from a human testis library. This new gene, the 5'end of which maps 10 kb from the translocation breakpoint, represents a strong candidate for MRX. Complete characterization of its structure and expression profile is underway, along with mutation analysis in MRX families with lesions mapping to Xq13.

Program Nr: 479 from the 1999 ASHG Annual Meeting

A simple method for generating target sequences for methylation-sensitive PCR. *O.A. Haas, A. Weinhäusel, R. Ebmer.* CCRI & LBICD, St. Anna Children's Hospital, Vienna, Austria (o.a.haas@magnet.at).

The analysis of differentially or abnormally methylated DNA becomes an increasingly important issue. It simplifies the diagnosis of specific constitutional disorders, such as syndromes associated with imprinting defects and uniparental disomies of particular chromosomes, and helps in the evaluation of neoplasm-associated methylation abnormalities. The most convenient and efficient laboratory method for this purpose is the methylation-sensitive polymerase chain reaction (MS-PCR). It takes advantage of the fact that chemical deamination of DNA with bisulfite converts unmethylated, but not methylated cytosine (C) to uracil which is subsequently replaced by thymidine (T). By that, unrelated DNA sequences are generated from originally homologous, but differentially methylated alleles. Since the respective PCR primers have to be designed according to the modified deaminated unmethylated and methylated DNA, the specific target sequence has to be defined unequivocally. So far, this was achieved by PCR amplification of an extended part of the deaminated sequence of interest, which thereafter had to be cloned and sequenced. Our approach is based on the assumption that in the unmethylated gene regions all CpG's are unmethylated, whereas in the methylated one all CpG's are methylated. We therefore simply deduce the sequence of the deaminated unmethylated DNA by converting all C's to T's. In the case of deaminated methylated sequence, we only change C's that are not contained in CpG's. To simplify this approach even further, we have developed a computer programme that generates the target sequence according to the above specification. With this method, we have already established and successfully applied diagnostic MS-PCR tests for the following genes: HUMARA, XIST, FMR1 (promoter and repeat), PEG1/MEST, SNRPN, BRCA1, ABL, RET and WT1.

Program Nr: 480 from the 1999 ASHG Annual Meeting

Digital Gene Localization of Bone-related ESTs. *L. Jia[1], N. Ho[1], J. Powell[2], L. Yang[2], P. Robey[3], M. Young[3], C. Francomano[1]*. 1) Medical Genetics Branch, National Human Genome Research Institute; 2) Center for Information Technology; 3) National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD. U.S.A.

Approximately 10,000 expressed sequence tags (ESTs) from 10 bone-related cDNA libraries have been analyzed against human High Throughput Genomic Sequence (HTGS) Database. This analysis, which has been termed digital gene localization (DGL), can predict the chromosomal location of the ESTs. We used the NCBI Network BLAST analysis with high stringency parameters (> 100 bp long, 98% homology) for the DGL. From this analysis, 407 ESTs from 10 human bone related cDNA libraries have assigned chromosomal locations. By radiation hybrid mapping and FISH, the chromosomal locations of 4 novel genes have been confirmed which are fully matched with the DGL results. The DGL analysis also predicted the gene structure, including intron/exon junctions, of these genes. We have calculated the bone-related gene distribution vs. whole UniGene distribution. Compared to the traditional gene localization methods, including FISH and radiation hybrid mapping, DGL is more accurate and faster, but limited to regions of the genome for which high throughput genomic sequence is available. DGL can greatly accelerate the chromosomal assignment of many genes.

Program Nr: 481 from the 1999 ASHG Annual Meeting

Complete genomic sequence of the 471 kb Familial Dysautonomia candidate region on chromosome 9q31. *M. Leyne[1], J. Mull[1], S.P. Gill[1], C.B. Liebert[1], C.M. Robbins[2], H.W. Pinkett[2], I. Makalowska[2], C. Maayan[3], A. Blumenfeld[3], F.B. Axelrod[4], M. Brownstein[2], B.P. Chadwick[1], J.F. Gusella[1], S.A. Slaugenhaupt[1]*. 1) Harvard Inst. of Human Genetics, Mass. General Hospital, Boston, MA; 2) National Institute of Health, Bethesda, MD; 3) Hadassah University Hospital, Jerusalem, Israel; 4) New York University Medical Center, New York, NY.

Familial Dysautonomia (FD) is an autosomal recessive disorder that affects the development and survival of sensory, sympathetic and some parasympathetic neurons. The gene defect is almost exclusively limited to the Ashkenazi Jewish population where the carrier frequency is 1:30. The FD gene maps to human chromosome 9q31 and recent haplotype analysis of 441 disease chromosomes has refined the candidate interval to a 471 kb region. A detailed physical map spanning this interval was constructed and consists of 3 overlapping BAC clones and 58 cosmids. We have used exon trapping and cDNA selection to identify 8 candidate genes in the FD region. In order to refine the candidate interval and isolate new FD candidate genes, we determined the genomic sequence of the 471 kb critical region by direct sequencing of the BAC clones. The genomic sequence has yielded several new polymorphisms that have narrowed the candidate region from 471 kb to 162 kb. In addition, gene prediction programs identified three new FD candidate genes. In total, 8 of the 11 candidate genes isolated to date map within the new 162 kb critical region.The genomic structure of these genes has been determined and mutational analysis of the coding sequence of these candidate genes was performed by sequencing RT-PCR products from affected and control individuals. No pathogenic mutations were identified within the coding regions of any of our candidate genes suggesting that the FD mutation may be located in an intron or in an untranslated region of one of these genes. A cosmid library from an FD patient homozygous for the FD haplotype has been constructed and we are currently constructing an FD contig of our candidate region. Direct sequence analysis of the FD contig sequence compared with the control contig sequence will allow us to identify the mutation resulting in FD.

Program Nr: 482 from the 1999 ASHG Annual Meeting

Evidence from single-nucleotide polymorphisms (SNPs) for natural selection affecting human and orangutan Xq28. *R.D. Miller, P. Taillon-Miller, P.-Y. Kwok.* Division of Dermatology, Washington Univ. Sch. Medicine, St. Louis, MO.

In the course of identifying informative SNPs on the long arm of the human X chromosome, we found and further investigated a 1.3 mb region which has fewer SNPs. This region, near the tip in Xq28, is rich in genes, among others containing those encoding the MMP1A homologue, creatinine transporter (CREAT), adrenoleukodystrophy protein (ALD), isocitrate dehydrogenase gamma (IDH), arginine-vasopressin receptor (AVPR2), renin-binding protein (RBP), host cell factor C1 (HCFC1), red cone photoreceptor (RCP), filamen A (FLN1), emerin (EMD), and glucose-6-phosphate dehydrogenase (G6PD). This region has an unusually high content of G+C (56% compared with 37% in Xq25). Conceivably, the reduced frequency of SNPs could be due to either a reduced mutation rate in this region or to one or more natural selection events. The prediction was that if there were a reduced mutation rate, a related species would have reduced divergence in this region, but if selection had occurred, the divergence in this region would be the same as in controls regions. To distinguish these alternatives, the comparative DNA sequence was determined from sequence tagged sites (STSs) from human and a female Sumatran orangutan. In the Xq28 region, more than 17,000 bp of homologous sequence from 26 STSs was obtained, and for comparison of divergence, additional homologous sequence was obtained from Xq25 and two nearby regions. In Xq28, the divergence between human and orangutan was 2.9% (standard deviation 0.95%), not significantly different from the divergence in Xq25 of 2.5% (probability = 0.25), nor from the two other regions. These results suggest that human Xq28 has been subjected to selection, reducing the frequency of informative SNPs. In this work, five heterozygous nucleotides were identified in the orangutan, none of which corresponded to human SNPs, and none were in the Xq28 region, suggesting that orangutans may also lack variability in this region.

Program Nr: 483 from the 1999 ASHG Annual Meeting

Determination of retrotransposition rates in cultured cells with a GFP-based assay. *E.M. Ostertag[1], E. Luning Prak[1], R.J. DeBerardinis[1], J.V. Moran[2], H.H. Kazazian, Jr.[1]*. 1) Dept Genetics, Univ Pennsylvania, Philadelphia, PA; 2) Dept Human Genetics, Univ Michigan, Ann Arbor, MI.

L1 retrotransposons are autonomous retroelements of which an estimated 40-60 remain active in the human genome. These elements occasionally create *de novo* insertions and can cause genetic disease. Here we describe a new retrotransposition assay using a Green Fluorescent Protein (GFP) retrotransposition marker and Fluorescence Activated Cell Scanning (FACS) analysis that is quicker and less subjective than our previous assay using a neomycin phosphotransferase (neo) marker. The new assay is able to detect near real-time retrotransposition in a single cell and is sufficiently sensitive to distinguish retrotransposition rates between different L1 elements. Therefore, this assay could be used for several important experiments such as determining dependence of retrotransposition on cell cycle and nuclear membrane breakdown, and demonstrating retrotransposition in a mouse model. In this study, we determine the retrotransposition rate of two different L1 retrotransposons, $L1_{RP}$ and L1.3. $L1_{RP}$ is an element recently discovered as a full-length *de novo* insertion into the RP2 gene of a retinitis pigmentosa patient. To date, $L1_{RP}$ and L1.3 are the two most active elements in the neo retrotransposition assay. The $L1_{RP}$ element retrotransposed in the GFP assay at a rate of approximately 0.3% of transfected cells per day, a rate about 5-fold higher than for L1..3. We also show that retrotransposition from an episomal vector can occur at a constant rate for at least 16 days in cultured cells. In summary, we have developed a new assay which demonstrates continuous and high frequency retrotransposition in HeLa cells and which we are using to develop a mouse model of retrotransposition.

Program Nr: 484 from the 1999 ASHG Annual Meeting

Centromere repositioning. *M. Rocchi, G. Montefalcone, S. Tempesta, R. Roberto, M. Ventura, N. Archidiacono.* Istituto Di Genetica, Universita Di Bari, Bari, Italy.

Primate pericentromeric regions have been recently shown to exhibit extraordinary evolutionary plasticity. In the present paper we report an additional peculiar feature of these regions which we discovered while analysing, by FISH, the evolutionary conservation of primate phylogenetic chromosome IV, IX, and X. If the position of the centromere is not included in the analysis, a relatively small number of rearrangements must be invoked to account for interspecific differences. However, if the centromere is included, a paradox emerges: the position of the centromere seem to have undergone, in some species, an evolutionary history independent from the surrounding markers. A significant number of additional rearrangements must be proposed to reconcile the order of the markers with centromere position. Alternatively, the evolutionary emergence of neocentromeres can be postulated. These data suggest that the evolutionary history of these regions is becoming increasingly more complex.

Program Nr: 485 from the 1999 ASHG Annual Meeting

Cross-species sequencing and functional studies identify non-coding elements with biological import including a potent IL-4 and IL-13 enhancer. *E.M. Rubin[1], G. Cretu[1], W. Miller[2], K.A. Frazer[1]*. 1) LBNL, Berkeley, CA; 2) Penn State Univ, University Park, Penn.

While computational methods for recognizing coding sequences are well established, greater than 95% of the human genome is comprised of non-coding sequences and we are currently faced with the challenge of shifting through these regions to identify non-coding elements of biological import. Based on the premise that biologically functional non-coding sequences will be conserved among vertebrates and that non-functional non-coding sequences will diverge, we compared an 830 kb region on human 5q31 which codes for 19 genes, including a bio-medically important cluster of cytokines genes, with the orthologous region on mouse chromosome 11. Following the identification of all the conserved sequences in this region and exclusion of the putative coding sequences we were left with 69 highly conserved (>100 bp and > 70% identity) non-coding elements. Experimental analysis of 15 of these elements demonstrated that they are single copy in the human genome and that 10 of them, in addition to humans and mice, are highly conserved in other vertebrates. The fact that these non-coding elements are present in multiple species suggests that they have been evolutionary conserved due to biological function. We have focused on assigning biological function to the most highly conserved non-coding element identified in these studies (401 bp in length and 83% identity) which is located in the ~12 kb region between IL-4 and IL-13 in the human, mouse, baboon and dog genomes. To study the function of this element we used a 450 kb 5q31 human YAC and the Cre-Lox system to create identical lines of human YAC transgenic mice differing only in that some contained and others lacked the 401 bp element on the YAC. Comparing the expression of the human transgenes in these different lines of mice revealed that the animals lacking the 401 bp element had a 10 fold decrease in expression of IL-4 and IL-13. These studies illustrate a cross-species sequence based strategy for identifying non-transcribed elements of biological import and have lead to the discovery of a potent regulator of IL-4 and IL-13 transcription.

Program Nr: 486 from the 1999 ASHG Annual Meeting

Possible activation of a b**-tubulin pseudogene (TUB4q) could explain the FSHD molecular defect.** *M. van Geel[1], A.F. Beck[1], E.E. Eichler[3], R.R. Frants[2], P.J. de Jong[1]*. 1) Dept of Cancer Genetics, Roswell Park Cancer Inst, Buffalo, NY; 2) MGC-Dept of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands; 3) Dept of Genetics, Case Western Reserve School of Medicine, University Hospitals of Cleveland, Cleveland OH.

The subtelomere of chromosome 4q35 contains the gene for the autosomal dominant neuromuscular disorder facioscapulohumeral muscular dystrophy (FSHD). Deletion of integral copies of a 3.3 kb repeated unit (D4Z4) are causal to the disease, but no associated gene has been identified. A b-tubulin related sequence, TUB4q, resides in the region. Functionality of the putative gene is questionable in view of demonstrated lack of expression. Nine family members of TUB4q have been identified mainly on pericentromeric and telomeric regions of the genome and originate from large segment duplications of Chr. 4q35. Like TUB4q, members on Chr. 10, 16 and 18 maintain an open reading frame and share close sequence relationships. To unravel the TUB4q family phylogeny, orthologous (derived from speciation event) and paralogous (derived from duplication event) TUB4q sequences were isolated from the chimpanzee (Pan) and baboon primate species. Besides the Chr. 4q35 TUB4q orthologue, 11 individual family members were identified, each in Pan and baboon. Both maximum likelihood and maximum parsimony methods were used to define orthologous and paralogous relationships among the human, baboon and Pan TUB4q copies. The putative baboon protein sequence is 88.7 % identical to the human Chr. 4 TUB4q orthologue, but surprisingly 97.1 % identical to the human Chr. 10 copy. Although, baboon and human diverged approximately 25 million years ago, TUB4q protein conservation remains, suggesting a functional TUB4q gene on human Chr. 10. Phylogenetic analysis suggests recent inactivation of the human Chr.4 TUB4q gene, which has been replaced by a duplicated copy on Chr. 10. Possible activation of the normally non-functional TUB4q gene in the FSHD region could have implications for the FSHD disease mechanism.

Program Nr: 487 from the 1999 ASHG Annual Meeting

Human BAC Ends. *S. Zhao[1], J. Malek[1], G. Mahairus[3], L. Fu[1], B. Nierman[1], C. Venter[2], M. Adams[2].* 1) Dept Eukaryotic Genomics, Inst for Genomic Research, Rockville, MD; 2) Celera Genomics Corp., Rockville, Maryland; 3) Dept Molecular Biotechnology, Univ of Washington, Seattle.

End sequences from Bacterial Artificial Chromosomes (BACs) have been playing critical roles in large scale genomic sequencing projects: 1) selecting clones for sequencing, 2) validating, joining and ordering contigs, and 3) building genome assembly scaffolds. To date, we have generated >300,000 end sequences from >180,000 human BAC clones with an average read length 460 bp for a total of 140 MB covering ~4.6% of the genome. Over 60% of the clones have BAC end sequences (BESs) from both ends representing >5.5X coverage of the genome by the paired ends clones. Our quality assessments and sequence analyses indicate that BESs from human BAC libraries developed at California Institute of Technology (CalTech) and Roswell Park Cancer Institute (RPCI) have similar properties. The analyses also indicate that BESs generated by The Institute for Genomic Research (TIGR) and the University of Washington (UW) are sufficiently accurate for use in both building the minimum tiling path of sequence-ready clones across the genome and building genome assembly scaffolds. The annotation results of BESs for the contents of available genomic sequences, sequence tagged sites (STSs), expressed sequence tags (ESTs), protein encoding regions and repeats indicate that this resource will be valuable in many areas of genome research.

Program Nr: 488 from the 1999 ASHG Annual Meeting

A High Level of Negative Meiotic Recombination Interference across a Large Region of Human Chromosome 17 Detected by Analyzing Single Sperm. *Y. Zou[1,2], X. Cui[1], C. Jackson-Cook[2], H. Li[1].* 1) Coriell Institute for Medical Research, 401 Haddon Avenue, NJ 08103; 2) Department of Human Genetics, Virginia Commonwealth University, Richmond, VA. 23298.

To learn the content of uneven distribution of meiotic recombination events in the human genome, four polymorphic markers that are located in TP53, VNT (vitronectin), B-Box (IAI3B), and Col1A1 (type I collagen, alpha 1) genes and span about a half portion (~51.9 cM on GeneMap'99 but 67.0 cM determined by the present study) of the human chromosome 17 were selected. Meiotic recombination frequencies between these markers were determined by analyzing single sperm from two donors. An excess number of double crossovers with respect to all possible tri-locus combinations of the four loci were detected. The coincidence coefficients ranged from 1.57 to 2.76 when the data for the two individuals were analyzed separately and from 1.67 to 2.41 for the pooled data. Such a high degree of negative interference could be a reflection of the physical structure favoring the occurrence of double crossovers in the covered chromosomal region. If the recombination events occurring during meiosis and mitosis share similar molecular mechanisms, these results may have a significant implication on understanding the frequent occurrence of loss of heterozygosity through an "interstitial deletion-like" mechanism as detected in a number of tumors. Results from the present study also indicate no significant difference in meiotic recombination frequencies between the two selected donors for all the intervals between the selected markers. However, two of the three intervals (between VNT and B-Box, and between B-Box and Col1A1) determined in the present study were shown to be significantly larger than reported previously. These results suggest a necessity of detailed genome-scale survey for understanding the genetic structure of the human genome.

Program Nr: 489 from the 1999 ASHG Annual Meeting

The molecular basis of late onset very-long-chain acyl-CoA dehydrogenase (VLCAD) deficiency. *B.S. Andresen[1,2], S. Olpin[3], M. Pourfarzam[4], T.J. Corydon[2], H. Lund[1,2], J. Schaefer[5], L.D. Schroeder[1,2], L. Bolund[2], N. Gregersen[1]*. 1) Research Unit for Molecular Medicine, Skejby Sygehus, Aarhus, Denmark; 2) Institute of Human Genetics, Aarhus University, Denmark; 3) The Children`s Hospital, Sheffield, UK; 4) Royal Victoria Infirmary, Newcastle, UK; 5) University Hospital of Dresden, Dresden, Germany.

VLCAD deficiency (VLCADD) is a potentially fatal defect of mitochondrial fatty acid -oxidation. Patients present with three different clinical phenotypes: Two childhood forms (severe and mild) and a late onset, exclusively muscular, form. We have investigated the molecular-genetic basis for the disease in eleven unrelated families with late onset VLCADD and compared this group of patients to a group of patients with severe childhood VLCADD. Mutant proteins were examined by overexpression of recombinant protein in COS cells. Patient cells were examined by beta-oxidation activity measurements and by tandem MS. Our results show that differences in the clinical presentation of VLCADD can be explained by differences in the molecular pathology of patient mutations, and that aberrant folding, leading to decreased amounts of protein, is a major molecular defect mechanism. We speculate that variation in the factors that influence folding and degradation of mutant proteins may explain some of the clinical variation observed. Finally, we present evidence that the CMT1A duplication (17p11.2) may be involved in disease in at least one late onset VLCADD patient.

Program Nr: 490 from the 1999 ASHG Annual Meeting

Molecular, biochemical and structural analysis of ten novel lysosomal neuraminidase mutations: correlation with clinical severity in sialidosis. *E. Bonten, A. d.Azzo.* Genetics, St Jude Child Res Hosp, Memphis, TN.

Sialidosis is a severe neurodegenerative lysosomal disorder caused by structural lesions in the lysosomal neuraminidases gene. Type I sialidosis (normophormic) is a mild form of the disease usually diagnosed in the second decade of life and characterized by the cherry-red spot-myoclonus phenotype and progressive impaired vision, but absence of dismorphic features. Type II sialidosis is a severe form of the disease, which can be subdivided into three forms: (I) congenital or hydropic (in utero), (II) infantile (0-12 months), and (III) juvenile (2-20 years). All type II patients eventually develop progressive mucopolysaccharidosis-like features, including coarse facies, visceromegaly, dysostosis multiplex, vertrebal deformities and severe mental retardation. We have identified 12 mutations, 10 of which novel, in the mRNA and genomic DNA of 9 unrelated sialidosis patients and some of their parents. The majority of the mutations were base substitutions resulting in single amino acid changes. Five patients were compound heterozygotes, while the others were homozygotes. To study the biochemical properties of the individual mutant proteins we expressed their encoded cDNA's in COS-1 cells or deficient sialidosis fibroblasts. Transfected cells were analyzed for residual neuraminidase activity and subcellular localization of the mutant enzyme. Based on these biochemical studies we grouped the mutations in three distinct catagories. Five mutant proteins lacked residual neuraminidase activity and were not localized in lysosomes. Three lacked residual activity, but had weak lysosomal localization. In contrast, the remaining three had residual activity (30-60%) and clear lysosomal localization. Interestingly, only the first category carried mutations on residues that are conserved within the family of bacterial/mammalian neuraminidases. Furthermore, we could make a clear correlation between the impact of the mutation on the neuraminidase function and the disease phenotype. In future the assignment of mutations to one of the groups might be helpful for determining the clinical prognosis of sialidosis patients.

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Program Nr: 491 from the 1999 ASHG Annual Meeting

The human rBAT promoter and promoter mutations in cystinuria. *M. Boutros, P. Ong, I. Saadi, F. Hiou-Tim, C. Vicanek, R. Rozen, P. Goodyer.* Human Genetics, McGill University, Montreal,PQ, Canada.

Cystinuria is an inherited (1:7000) disorder of the kidneys and intestine, affecting reabsorptive transport of cystine and dibasic amino acids. Affected individuals develop recurrent kidney stones due to the low solubility of cystine in acidic urine. There are 2 known cystinuria loci. The rBAT gene (2p21) encodes a transmembrane glycoprotein expressed at the apical surface of cells in the proximal renal tubule and small intestine. Over 30 distinct rBAT coding mutations have been identified in association with the fully recessive (Type I) form of cystinuria. A second gene (19q) accounts for the incompletely recessive forms (Types II and III. To study developmental regulation and to search for promoter mutations, we isolated 4kb of the 5' rBAT flanking sequence. We noted five SP-1, four AP-2, one NF-kB, 11 gamma interferon sites and a potential PAX 8 site. We screened a panel of cell lines and found that the first 800 nucleotides of the rBAT promoter are sufficient to drive tissue- specific expression at 10-fold higher levels in a proximal tubular cell line. Co-transfection of PAX 8, a proximal tubule specific transcription factor expressed during development, with this 800 bp promoter showed a 2-fold increase in activity. To determine if rBAT is developmentally regulated, we performed Northern analysis on samples of kidney from a fetus (18 weeks) and a 3-year old girl. mRNA levels were 3-5 fold higher in post-natal tissue. Immunohistochemical analysis of human kidney tissue revealed that rBAT is expressed in the early and late segments of the proximal tubule. In a group of Type I/III patients, we found 2 promoter mutations: G(-265)A located within an AP-2 site, and G(-194)C. In transfection experiments, we found that G(-265)A decreases promoter activity to 60 percent of wild type. Studies of the second mutation are in progress.

Program Nr: 492 from the 1999 ASHG Annual Meeting

Glycerol Kinase Deficiency: Patients' Missense Mutations Map to Active Site Cleft. *K.M. Dipple[1,2], Y.H. Zhang[1,2], B.L. Huang[1,2], E.R.B. McCabe[1,2].* 1) Dept Pediatrics, Div Genetics, UCLA, Los Angeles, CA; 2) Mattel Children's Hospital at UCLA, Los Angeles, CA.

Glycerol kinase deficiency (GKD) occurs as part of an Xp21 contiguous gene syndrome or as isolated GKD. The isolated form of GKD may be either symptomatic (episodic metabolic and central nervous system decompensation) or asymptomatic (only pseudo-hypertriglyceridemia). To better understand the pathogenesis of isolated GKD, and attempt to determine genotype-phenotype correlations, we mapped six missense mutations to a model based on the crystal structure of E. coli GK (Hurley et al., Science 259:673, 1993). The use of the E. coli structure is justified based on the 50% identity and 65% similarity with human GK. Mutations were analyzed by automated genomic sequencing (ABI 377). Two of the patients (N288D and Q438R) were asymptomatic while the third (M428T) was difficult to classify because he had neonatal asphyxia, which could be a presentation of GKD, but was asymptomatic after the neonatal period. Only one of the patients (R405Q) was clearly symptomatic with episodic hyperglycerolemia, glyceroluria, and hypoglycemia. In addition to the patients we identified, two patients with missense mutations were reported in the literature and were asymptomatic (D440V: Walker et al., Am. J. Hum. Genet. 58:1205, 1996; W503R: Sjarif et al., J. Med. Genet. 35:650, 1998). The structure of E. coli GK has two large domains that are separated by a deep cleft that is the active site. Mapping of the patients' missense mutations to the structure of E. coli GK using the Insight II program, showed that all but one of the patients' missense mutations map to the active site cleft. The one mutation that was not in the active site cleft mapped adjacent to it. These data suggest that, with this small sample size, patients with isolated GKD due to missense mutations have alterations that map to the active site of GK. We are unable to distinguish the impact of the mutation in the obviously symptomatic patient (R405Q) from those in the asymptomatic patients. More detailed structural and function analysis of these mutations will be required to understand their precise roles in the pathogenesis of GKD.

Program Nr: 493 from the 1999 ASHG Annual Meeting

Molecular cloning, characterization and functional expression of a second mammalian delta1-pyrroline-5-carboxylate reductase. *K. Dougherty[1], C.A. Hu[2], C. Obie[2], D. Valle[2]*. 1) Peridontics Prevention and Geriatrics, Univ of Michigan, Ann Arbor, MI; 2) Institute of Genetic Medicine and the HHMI, Johns Hopkins University School of Medicine, Baltimore.

Mammalian P5CR is an ATP- and NAD(P)H-dependent enzyme catalyzing the reduction of P5C to proline, the first committed step in the de novo biosynthesis of proline. Biochemical studies of P5CR obtained from a variety of tissues have shown tissue-specific kinetic properties and sensitivity to inhibitors. To better understand the molecular enzymology, tissue-specificity and regulation of mammalian P5CRs, we previously isolated a human gene encoding P5CR (P5CR.1) by complementation cloning in S. cerevisiae (Dougherty et al., JBC 267:871, 1992). We have now identified a second human gene encoding a putative P5CR, designated P5CR.2. The P5CR.2 ORF is 960 bp with 81% nucleotide identity to P5CR.1, and encodes a protein with 84% amino acid identity to P5CR.1. Both P5CR.1 and P5CR.2 genes are broadly expressed. To confirm the identity of the putative P5CR.2 cDNA, we expressed it in a P5CR-deficient strain of S. cerevisiae and showed that, like P5CR.1, it conferrs proline prototrophy. In a search for human diseases associated with P5CR deficiency, we noticed that there is a proline auxotrophic human leukemic lymphoblastoid cell line (REH), previously shown to be deficient in NADH-dependent P5CR activity, but with normal NADPH-dependent P5CR activity (Lorans and Phang, BBRC 101:1018, 1981). Northern blot analysis and direct sequencing of RT/PCR product showed that the REH cells expressed a normal amount of wild-type P5CR.2 transcript, but undetectable P5CR.1 transcript. In conclusion, we have cloned and characterized a second human gene encoding P5CR and identified a cell line expressing only P5CR.2. This result together with earlier biochemical studies of P5CR activities in these cells indicate P5CR.2 utilizes NADPH while P5CR.1 utlizes NADH. We speculate that various combinations of expression of P5CR.1 and P5CR.2 accounts for the tissue variations in the biochemical characteristics of P5CR activity.

Program Nr: 494 from the 1999 ASHG Annual Meeting

Screening candidate genes in patients with Hermansky-Pudlak syndrome. *M. Huizing[1], V. Shotelersuk[1], E.C. Dell'Angelica[2], J.S. Bonifacino[2], W.A. Gahl[1].* 1) HDB; 2) CBMB, NICHD, NIH, Bethesda, MD.

Hermansky-Pudlak syndrome (HPS) is an autosomal recessive disorder characterized by oculocutaneous albinism, platelet storage pool deficiency, and ceroid lipofuscinosis. Although the basic defect in HPS is not known, it is considered to involve a vesicular membrane shared by the melanosome, dense body, and lysosome, all of which are affected in HPS. Several genes, some corresponding to mouse models manifesting both hypopigmentation and platelet storage pool deficiency, are candidates to cause HPS. The discovery of one HPS-causing gene, HPS-1, and its murine homologue ep (for pale ear), may lead us to the basic defect, but the function of the HPS1/ep protein product has not yet been determined. In addition to mutations in HPS-1, we demonstrated that mutations in the gene for the beta-3a subunit of adaptor complex-3 (AP-3) cause a variant of HPS in two brothers; AP-3 mutations also result in the HPS mouse called pearl. AP-3 is a coat protein that assists in vesicle formation from Golgi membranes. Other candidate genes for causing HPS include the genes responsible for murine models of the disease as well as genes involved in vesicular trafficking, cargo packaging, vesicle formation and docking, or membrane fusion. We used immunofluorescence microscopy followed by DNA-sequencing to screen HPS patients for defects in candidate genes. The platelets of 9 patients with mutations in HPS-1 showed dramatically decreased fluorescence when treated with anti-HPS-1 antiserum. This illustrates the utility of our HPS-1 antibody, and the feasibility of using immunofluorescence to ascertain patients with HPS-1 mutations. Similarly, fibroblasts of the beta-3a deficient patients showed decreased signal when incubated with beta-3a antiserum or with antiserum against the mu or delta subunits of the AP-3 complex. We also screened the fibroblasts of 27 patients without HPS-1 mutations for the presence of other candidate gene products, including ARF1, ARF6, rab27, and the mu subunit of AP-3. Although no abnormalities in these proteins were detected, immunocytofluorescence appears to be a reasonable technique for identifying abnormalities in protein trafficking causing HPS.

Program Nr: 495 from the 1999 ASHG Annual Meeting

Fabry Disease: Biochemical, Molecular and Subcellular Localization Studies of Eight a-**Gal A Missense Mutations with Residual Activity.** *Y.A. Ioannou, P. Ashton-Prolla, C.M. Eng, R.J. Desnick.* Human Genetics, Mount Sinai Sch Medicine, New York, NY.

Fabry disease is an inborn error of glycosphingolipid catabolism resulting from the deficient activity of the lysosomal hydrolase, a-galactosidase A (a-Gal A). PCR amplification and subsequent sequencing of genomic DNA from unrelated patients with residual a-Gal A activity (mild cardiac variants) identified eight missense mutations E59K, I91T, R112H, F113L, N215S, D244N, M296V and R356W. Of note, the D244N mutation created a new N-glycosylation consensus sequence. Each mutation was introduced by site-directed mutagenesis into the a-Gal A cDNA which was modified by addition of a FLAG octapeptide and a histidine tag. Using this transient expression system, the wild type and mutant a-Gal A cDNAs were expressed at levels 25-fold and 1.5 to 30-fold greater than endogenous COS-7 activity (0.15 mmoles/hr/mg), respectively. The expressed wild-type and mutant enzymes were characterized with respect to enzyme activity, pH stability and intracellular localization. Different disease-causing mechanisms were identified for these mutations including decreased affinity for both the natural and artificial substrates, retention in the endoplasmic reticulum, a delay in protein transport/maturation, and instability at acidic pH. In addition, when each missense mutation was expressed in COS-7 cells and incubated in the presence of 200 mM D-galactose for 36 hr, the cellular a-Gal A activities were increased by 1.3 to 5 fold greater than that of the respective mutation expressed in the absence of the substrate analogue. These results emphasized the molecular heterogeneity of the mutations causing Fabry disease and indicated that the residual activity in Fabry cardiac variants with residual activity can be increased by substrate analogues that facilitate proper enzyme folding and lysosomal transport.

Program Nr: 496 from the 1999 ASHG Annual Meeting

Metabolic consequences of targeted disruption of the 70kDa Peroxisomal Membrane Protein (PMP70). *G. Jimenez-Sanchez, K.J. Hebron, C. Obie, G. Thomas, D. Valle.* Institute of Genetic Medicine and The Howard Hughes Medical Institute, Johns Hopkins University, Baltimore, MD.

ATP-binding cassette (ABC) transporters are members of a superfamily of membrane proteins involved in the transport of a variety of molecules across biological membranes. There are 4 known human peroxisome ABC transporters: PMP70, ALDP, ALDP-related (ALDR) and PMP70-related (P70R). We targeted the murine PMP70 gene by standard methods. PMP70-/- animals lack PMP70 mRNA and protein but immunohistochemistry shows that peroxisomes are present and contain normal amounts of PTS1 and PTS2 targeted matrix proteins. Thus, PMP70 is not required in peroxisome biogenesis. Metabolic evaluation of these animals revealed dicarboxylic aciduria suggesting a defect in b-oxidation. Consistent with this, we found that PMP70-/- mice show cold intolerance with a decrease in body temperature to 12^{o}C after 90 min at 4^{o}C. Whether this impaired thermogenesis reflects impaired peroxisomal b-oxidation or a secondary defect in mitochondrial b-oxidation is currently under investigation. Immunoblots of PMP70-/- liver and kidney showed a specific massive increase (>20X) of P70R suggesting this may compensate for the absence of PMP70. Steady state levels of P70R mRNA are not different from control indicating the mechanism for P70R accumulation in PMP70-/- is post transcriptional. Preliminary pulse studies suggest that P70R is synthesized at a higher rate in the PMP70-/- mice suggesting some alteration in translational regulation accounts for P70R accumulation. PMP70 mice have a marked (10X) reduction in hepatic glycogen in the fed state. Glycogen synthase mRNA and activity levels are not different from control. The level of hepatic glycogen increases in animals on a high (69%) carbohydrate diet, confirming the ability of PMP70-/- mice to synthesize glycogen. Taken together these results suggest increased utilization of glycogen in the PMP70-/- mouse. How these metabolic abnormalities derive from deficiency of PMP70 is not clear but we favor a model in which impaired peroxisomal b-oxidation of dicarboxylic fatty acids is the primary defect.

Program Nr: 497 from the 1999 ASHG Annual Meeting

Canine model and genomic structure organization of glycogen storage disease type Ia (GSD Ia). *P. Kishnani[1], E. Faulkner[1], S. Vancamp[2], M. Jackson[2], T. Brown[2], A. Boney[1], Y.T. Chen[1]*. 1) Medical Genetics, Duke Univ Medical Center, Durham, NC; 2) North Carolina State Univ, NC.

GSD Ia was diagnosed in 6 pups from an established canine (maltease beagle) GSD Ia breeding colony. 5 pups died within 6 weeks (2 within minutes, 1 at 36 hrs, 1 each at weeks 4 and 6). 1 is alive at age 6 months. Histology of affected pups showed diffuse, marked hepatocellular vacuolation, with distended clear hepatocytes and central to marginally located rounded nuclei. Kidneys showed increased cellularity & finely granular eosinophilic expansion of mesangium in several glomelular tuffs as early as age 8 days which progressed to segmental/ diffuse increased cellularity with age. Vacuolation of proximal convoluted tubular epithelium was also noted. Biochemical analysis revealed increased liver glycogen content and isolated marked reduced glucose -6-phosphatase (G-6-Pase) enzyme activity in liver and kidney. Affected pups were homozygous for the M121I GSD Ia mutation identified in this breed. They have tremors, weakness and neurologic signs when hypoglycemic. They have retarded postnatal growth and development and progressive hepatomegaly. Chemistries include fasting hypoglycemia 20-40 (60-100mg/dl), increased blood lactate 4.2-11.5 (0.7-2.5 mg/dl), cholesterol 326-639 (102-173 mg/dl), triglycerides 33-142 (2-26 mg/dl) and uric acid 0-1.9 (0-0.3 mg/dl). Treatment includes frequent PO feeds, which provide 12-14 mg/kg/min glucose. We have characterized the canine G-6-Pase gene by screening a canine genomic library with our published canine G-6-Pase cDNA as probe. It spans ~11.8kb and consists of 6 exons (> 90% amino acid sequence homology to derived human sequence). We have sequenced the first 1.4kb of the 5' region. It contains several putative response element motifs homologous to the published human 5' region (GRE, IRS, HNF 1, HNF 5,C/EBP, CRE, AP1, and AP2 sites). Putative TATAA and CAAT promoter boxes are also present. Establishment of this GSD Ia canine colony, with affected dogs that closely resemble human disease makes it a perfect model to better study pathophysiology, long term complications and to develop novel therapeutic approaches such as drug and gene therapy.

Program Nr: 498 from the 1999 ASHG Annual Meeting

Analysis of the expression of glutaryl-CoA dehydrogenase: In vitro and In vivo studies. *D.M. Koeller, M. Woontner, J. Kwon, L.S. Crnic.* Dept Pediatrics, Univ Colorado Health Sci Ctr, Denver, CO.

Glutaric acidemia type I (GAI) is an autosomal recessively inherited organic acidemia caused by a mutation in the glutaryl-CoA dehydrogenase(GCDH)gene. Glutaryl-CoA dehydrogenase catalyses the oxidative decarboxylation of glutaryl-CoA, an intermediate in the oxidation of lysine and tryptophan. Clinically, the most characteristic feature of GAI is progressive dystonia. The pathophysiologic basis for the movement disorder seen in GAI patients is degeneration of neurons in the caudate and putamen nuclei of the striatum. In an attempt to understand the basis for the specific neuropathology in GAI, we have analyzed the expression of the GCDH gene using both in-vitro and in vivo approaches. Transfection studies in fibroblasts and hepatocytes mapped the mouse GCDH promoter within a 400 bp region of DNA 5' of the translation start site. The promoter lacks a TATA consensus sequence, but includes a potential CCAAT box, and several Sp1 sites. An 11 bp element important for promoter activity was identified in both the mouse (2 copies) and human (1 copy) GCDH promoters. Western blot analysis of mouse tissues demonstrated that the highest levels of GCDH expression are in liver and kidney, consistent with its role in amino acid oxidation. Expression in multiple regions of the brain was also detected by western blotting. Analysis of mice expressing a beta-galactosidase reporter gene under the control of the GCDH promoter demonstrated expression in the hippocampus, the cerebral cortex, and both granule and pyramidal cells in the cerebellum. Significantly, no expression of the GCDH promoter driven beta-galactosidase gene was detected in the caudate-putamen, the region of the brain most severely affected in GAI patients. Based on these results, possible models for the pathophysiology of GAI are presented.

Program Nr: 499 from the 1999 ASHG Annual Meeting

The Hermansky-Pudlak syndrome (HPS) protein is a component of a tissue-specific organellar protein trafficking complex. *J. Oh[1], Z.-X. Liu[1], G. Raposo[2], R.A. Spritz[1]*. 1) Human Medical Genetics Program, Univ Colorado Health Sci Ctr, Denver, CO; 2) Institut Curie, Paris, France.

Hermansky-Pudlak syndrome (HPS) is an autosomal recessive disorder in which oculocutaneous albinism, bleeding, and ceroid-lipofuscin lysosomal storage disease are associated with defective biogenesis of multiple cytoplasmic organelles: melanosomes, platelet granules, and lysosomes. The HPS protein is a novel 700-aa protein that is unrelated to other known proteins and which contains no peptide motifs that might provide clues to its function. Furthermore, analysis of HPS patient mutations and comparison of the human, mouse, and rat HPS polypeptides fails to highlight obvious critical, conserved, or divergent segments of the protein. We present biochemical, cell biological, immunological, and microscopic data that suggest that HPS protein is a component of a tissue-specific protein complex involved in trafficking of "cargo proteins" destined for nascent organelles. Biochemical and cell fractionation studies show that the HPS protein is a non-glycosylated, non-membrane cytoplasmic protein which in non-melanotic cells is contained principally in a ~200 kDa protein complex located in the cytosol and in a small vesicle fraction. In contrast, in melanotic cells about half of the HPS protein is contained in a >400 kDa membrane-associated protein complex. Confocal and digital microscopy show that this cell-specific complex is located in the perinuclear region, and by immunoelectron microscopy this compartment appears to consist of small uncoated peri-Golgi vesicles and early-stage melanosomes. However, the HPS protein is not associated with the trans-Golgi network, and co-immunoprecipitation analyses show that HPS is not directly associated with the AP-3 adaptin-like complex, which has been implicated in both cargo protein trafficking and in HPS-like clinical features. We suggest that the function of the HPS protein may be to receive cargo proteins from AP-3 or other components of the cargo protein sorting machinery, and deliver these proteins into specific nascent organelles.

Program Nr: 500 from the 1999 ASHG Annual Meeting

Hyperimmunoglobulinemia and periodic fever syndrome due to mutations in the gene mevalonate kinase (MVK). *B.T. Poll-The[1], S.M. Houten[2], J. Frenkel[3], W. Kuis[3], M. Duran[1], T.J. de Koning[1], A. van Royen-Kerkhof[3], G.J. Romeijn[2], L. Dorland[1], G.T. Rijkers[3], H.R. Waterham[2], R.J.A. Wanders[2].* 1) Dept Metabolic Disorders, Univ Children's Hosp, Utrecht, Netherlands; 2) Clinical Chemistry and Pediatrics, Academic Medical Centre, University of Amsterdam, Netherlands; 3) Dept. of Immunology, Wilhelmina Children's Hospital, Utrecht Medical Center, Netherlands.

Hyperimmunoglobulinemia D and periodic fever syndrome (HIDS) is characterized by recurrent episodes of fever, lymphadenopathy, arthralgia, gastrointestinal symptoms and rash. During attacks of severe fever, mildly increased excretion of mevalonic acid (5.3 to 27.8 mmol per mole creat) was noticed in HIDS. Mevalonate kinase (MK) activity was 1.3 - 6.5% of the mean in control lymphocytes, and 0.8 - 6.1 % of the mean in control fibroblasts. Deficient MK-activity and increased excretion of mevalonic acid is also found in mevalonic aciduria, a severe multisystemic disorder associated with periodic fever. However, in these patients the excretion of mevalonic acid is constitutive and much higher than observed in HIDS. Sequence analysis of MVK cDNAs identified the same 1129 G -> A mutation in all 8 patients currently examined. Two patients were homozygous for this mutation, which changes the valine at position 377 of the MK sequence to isoleucine (V377I). Six patients were compound heterozygotes for the 1129 G -> A mutation and an additional missence mutation: 803 T -> C, I268T (3 patients); 59 A -> C, H20P (2 patients); 116 T -> C, L39P. None of 24 sequenced control subjects carried the V377I allele. The identification of the 1129 G -> A mutation in 8 unrelated HIDS patients suggests that this allele is responsible for the HIDS presentation. Immunoblot analysis demonstrated a deficiency of MK in patients' cells indicating a protein destabilization effect of the mutations. Our findings in patients with a dysregulation of the immune system expand the phenotypic spectrum of MK deficiency, and demonstrate an interrelation between the cholesterol biosynthetic pathway and the regulation of the immune response.

Program Nr: 501 from the 1999 ASHG Annual Meeting

Carbamyl phosphate synthetase I (CPSI) deficient patients express only mutant allele message despite genomic heterogeneity. *M. Strand[1], L. Hall[2], M. Summar[2]*. 1) Pediatrics, Vanderbilt University, Nashville, TN; 2) Pediatrics, Medical Genetics, Vanderbilt University, Nashville, TN.

In our studies of patients with CPSI deficiency (CPSID), our sequence analysis of CPSI message identified only one unique mutation for each patient (19 of 20 patients). The sequence did not show either a normal band or a different mutation elsewhere. The sequence analysis was performed on fragments derived from patient RNA isolated from liver, fibroblast, or lymphoblastoid cells. If the RNA reflects transcription from both CPSI alleles, this implies that all patients carry the same unique mutation on both chromosomes. In the case of rare metabolic diseases, a high degree of consanguinity is expected, which often explains such homozygosity. However, when we analyzed the genomic DNA for a common CPSI exonic polymorphism we found 6 of the 12 patients were heterozygous. We have subsequently analyzed 6 patients and found that in 4 cases the genomic DNA is heterozygous for their unique mutations. Southern blot analysis of these patients does not reveal a large deletion and we have not identified patients without expressed CPSI message. These results suggest that a common null mutation resulting in loss of expression for one allele does not explain this phenomenon. This observation may represent imprinting of one of the CPSI alleles rendering it silent for expression. Such a situation will impact the prenatal diagnosis of this metabolic disorder and our understanding of the tight regulation of this gene.

Program Nr: 502 from the 1999 ASHG Annual Meeting

A mouse model for the Smith-Lemli-Opitz syndrome (SLOS). *G.S. Tint[1,2], H. Waage[3], K.K. Sulik[3], F.F. Moebius[4], B.U. Fitzky[4], H. Glossmann[4], S. Shefer[2], G. Salen[1,2]*. 1) VA New Jersey Health Care System, East Orange, NJ; 2) UMDNJ-New Jersey Medical School, Newark, NJ; 3) University of North Carolina, Chapel Hill, NC; 4) Institute for Biochemical Pharmacology, Innsbruck, Austria.

SLOS is a not-uncommon severe birth defect-mental retardation syndrome caused by a defect in 7-dehydrocholesterol (7DHC) D7-reductase (DHCR7. E.C.1.3.1.21), the final enzyme in the cholesterol (CH) biosynthetic pathway. Children exhibit multiple abnormalities of face, limbs, genitalia and internal organs, develop slowly, are photosensitive, frequently exhibit destructive behaviors and have greatly reduced survival. Human DHCR7 contains 9 exons, spans 14 kb, is localized to 11q13 and codes for a 55 kDa protein. Using stem cell gene targeting we mutated this gene in the mouse to create an animal model for the syndrome. Matings of animals heterozygous for the targeted disruption have thus far resulted in 11 -/-, 20 +/-, and 7 +/+, which approximates the 1:2:1, expected ratio. The male to female ratio is also approximately as expected. The litters were of normal size, indicating that all pups survive to term. However, the homozygotes died shortly after birth (within about 14 hrs). Among the eleven -/- mice, two wide cleft palates were observed while abnormally distended bladders were also noted in 6 -/- mice predominately in males. DHCR7 activity in 8 -/- newborn mice was found to be ~ 1% of that in a control animal (26±20 vs. 2030pmol/min/mg protein). Liver 7DHC concentrations, undetectable in controls, were 0.43±0.24 mg/g in 8 -/- animals while CH levels averaged 0.69±0.50 mg/g (compared to 1.90±0.66 mg/g in 7 adult controls). In brain (n=3 -/-) the defect was far worse with mean CH and 7DHC levels of 0.41±0.03 and 1.19±0.23 mg/g, respectively, vs. 3.99 and <0.01 mg/g, respectively, in 2 +/- newborns. All of the biochemical parameters closely mirror the differences we see between the group of affected SLOS children who are in the middle range of clinical severity and age matched controls. Thus, the model appears to successfully model CH and 7DHC metabolism in about 50% of affected children.

Program Nr: 503 from the 1999 ASHG Annual Meeting

Paradoxical effect of acid b**-galactosidase gene dosage on the phenotype of twitcher mouse (genetic galactosylceramidase deficiency).** *J. Tohyama[1], M.T. Vanier[2], K. Suzuki[1], T. Ezoe[1], J. Matsuda[1], K. Suzuki[1]*. 1) University of North Carolina, Chapel Hill, NC; 2) INSERM U189, Lyon-Sud School of Med, Oullins, France.

Twitcher is a mouse model of human Krabbe disease (globoid cell leukodystrophy) due to a mutation in the galactosylceramidase gene. We recently generated a mouse model of human GM1-gangliosidosis by inactivating the acid b-galactosidase locus. These mutants have been cross-bred to evaluate the genetic and metabolic relationship between the two lysosomal b-galactosidases. Comparisons were made only among offspring of different genotypes from double-heterozygote mating. The mice doubly deficient for both b-galactosidases develop massive accumulation of lactosylceramide in all tissues, as predicted from the known overlapping substrate specificity of these enzymes. However, different acid b-galactosidase gene dosage have dramatic and counter-intuitive effects on the phenotype of twitcher mice that defy conventional wisdom concerning autosomal recessive genetic disorders. Paradoxically, twitcher mice with additional complete acid b-galactosidase deficiency have by far the mildest clinical and neuropathological phenotype with the longest life span, followed by twitcher mice with a normally active pair of acid b-galactosidase genes. Furthermore, twitcher mice with a single functional acid b-galactosidase gene have the most severe disease with the shortest life span and additional hyper-reactivity and seizures not seen in other genotypes. Consistent with the clinical seizures, these twi -/-, GM1 +/- mice develop neuronal degeneration, particularly in the CA3 region of hippocampus and superficial layers of cerebral cortex, and less prominently in basal nuclear structures. Brains of the doubly deficient mice inexplicably contain only half normal amounts of galactosylceramide throughout their development despite the relative preservation of myelin. Brain psychosine (galactosylsphingosine) levels are dramatically increased in all twi -/- mice irrespective of the acid b-galactosidase gene dosage. These findings indicate that there are hitherto unrecognized genetic and metabolic interrelationships between the two lysosomal b-galactosidases and among their substrates.

Program Nr: 504 from the 1999 ASHG Annual Meeting

A cost-effective, time-efficient scanning strategy for genome scans of recessive diseases. *J.S. Acierno Jr[1,2], J.F. Gusella[1,2], S.A. Slaugenhaupt[1,2]*. 1) Harvard Institute of Human Genetics, Harvard Medical School, Boston, MA; 2) Genomics Core Facility, Massachusetts General Hospital, Charlestown, MA.

Researchers are often faced with two obstacles when beginning a genome scan for linkage studies: (1) delay of the start of a genome scan due to the initial availability of DNA on only a single multi-affected family, with continued collection of additional families expected, and (2) the prohibitive cost of completing a scan on the large number of individuals needed to obtain linkage.

For recessive diseases with no phenocopies, an initial genome scan of a single nuclear family of parents with at least two affected children can exclude a substantial amount of the genome by identifying broad regions of chromosomes through haplotyping in which the affected offspring are not sharing both parental chromosomes. The availability of grandparents can increase the amount of exclusion by establishing phase for regions not completely informative in the parents. Unaffected siblings can also increase exclusion through identifying regions they share with their affected siblings.

We propose a novel strategy of completing an initial screen on a single two- or three-generation nuclear family with multiple affected children followed by haplotype analysis for identification of discordance. This subset of remaining markers can then be used for a linkage study once adequate families are collected. We then illustrate the practical application of this strategy through describing a disease recently linked by our institution using this method and the resulting substantial reduction in time to completion and number of genotypes performed. This strategy can reduce the total amount of genotypes performed by up to approximately 75% through genotyping as few as 4 or 5 individuals.

Program Nr: 505 from the 1999 ASHG Annual Meeting

EXPERIMENTAL DETERMINATION OF THE EFFICIENCY OF HAPLOTYPE CONSTRUCTING PROGRAMS USING 5 SNPS FROM THE NAT2 GENE. *C.J. Allan, K. CANTONE, P. Khan, P. Boyd, I. Purvis, C.-F. Xu.* Discovery Gen, Molec Gen Unit, Glaxo Wellcome, Hertfordshire, England.

Haplotype analysis has been a powerful tool in narrowing down the location of disease susceptibility genes. In addition to its use in linkage studies in pedigrees, haplotype analysis has been applied to population studies and can be used in determining the origin and migration of ancestral alleles. Several computer programs are available which can construct haplotypes from the genotypes of unrelated individuals. The present study has used polymorphisms in the NAT2 gene as a model system to evaluate the efficiency and accuracy of these programs. The genotypes of 80 individuals for each of 5 SNPs within an 850bp PCR fragment were determined by direct sequencing. Haplotypes were subsequently estimated by a program using an E-M algorithm written by Chiano and Clayton. In parallel, the PCR products from the same individuals were cloned into TA cloning vectors and the haplotypes of each individual were experimentally determined by sequencing 8-10 clones. A comparison of the 2 sets of haplotype data showed a 99.9% match between the computer generated haplotypes and those determined experimentally. Our results therefore suggest that the E-M algorithm based haplotype prediction program can provide effective estimates of haplotypes for unrelated individuals using genotype data from multiple SNPs.

Program Nr: 506 from the 1999 ASHG Annual Meeting

Missense mutations of the TLR4 gene are associated with hyporesponsiveness to lipopolysaccharide (LPS) in humans. *N.C. Arbour[1], B.C. Schutte[2], J.N. Kline[1], K.L. Frees[1], S.J. Swartz[1], N.C. Meyer[1], D.A. Schwartz[1,3].* 1) Dept of Medicine; 2) Dept of Pediatrics; 3) Veterans Affairs Medical Center, University of Iowa, Iowa City, IA.

Bacterial LPS or endotoxin causes the adverse effects of Gram negative sepsis and influences the course of acute respiratory distress syndrome, asthma, and other forms of chronic airway disease. We recently showed that healthy non-asthmatics demonstrate a reproducible airway response to inhaled LPS, with some developing airflow obstruction at low LPS concentrations and others unresponsive to high LPS levels. The Toll-like receptor TLR4 has been implicated in LPS signaling and a missense mutation identified in the mouse TLR4 gene that is associated with a hyporesponsive LPS phenotype. To test whether mutations in the TLR4 gene are associated with LPS hyporesponsiveness in humans, we developed a single stranded conformational polymorphism assay in which the coding region of the TLR4 gene was screened for mutations in 83 normal, healthy subjects challenged with incremental doses of endotoxin. Band variants were confirmed by sequencing and amplification-refractory mutation system analyses. We identified two missense mutations (Asp299 Gly and Thr399 Ile) within the fourth exon, both of which are located in the extracellular domain of the TLR4 receptor. Asp299 is highly conserved between species but Thr399 is not. The allelic frequency of the two substitutions was approximately 6.6% in our study population, 7.9% in a normal control population from Iowa, and 3.3% in CEPH parental chromosomes. 22.6% of the study subjects who were hyporesponsive to inhaled LPS had this sequence variant while only 5.8% of the LPS responsive subjects had this sequence variant (P=0.029). These results provide the first genetic link between a gene associated with LPS signaling and reduced response to LPS in humans. This finding contributes to our overall understanding of LPS signaling and may have important implications for the treatment of Gram negative sepsis as well as other diseases caused by exposure to endotoxin.

Program Nr: 507 from the 1999 ASHG Annual Meeting

Epistatic interactions and mapping of loci that modify the severity of PKD in a mouse model. *D.R. Beier, S. Kuida, O.A. Iakoubova.* Div Genetics, Brigham & Women's Hosp, Harvard Med Sch, Boston, MA.

Mouse models of human disorders are powerful tools for identifying modifying loci that influence complex genetic traits. We have previously identified modifiers that mediate the severity of PKD in the juvenile cystic kidney *(jck)* mouse using F2 progeny of a C57BL/6 x DBA/2 (B6xD2) intercross. Specifically, we found a highly significant association for the inheritance of B6 alleles on chr 1 with severe disease. This observation was unexpected, since the PKD phenotype in the B6 background is not severe. We proposed that the inheritance of both the B6 locus on chr 1 and a D2 gene elsewhere in the genome results in the severe phenotype, presumably as a consequence of an interaction.

We have now proven that the severe disease phenotype is a recessive trait, since F1 *jck/jck* mice have very mild PKD. When heterozygous mice from this cross are intercrossed, the F2 progeny recapitulate the variable phenotype seen in the previous B6xD2 intercross, supporting the model that an interaction of parental alleles causes severe PKD. Given that the chr 1 locus behaves recessively, the statistical effect of the interacting locus will be obscured in an intercross, because only 25% of the progeny are informative for the interaction. To address this, we made a D2.B6 *jck/+* congenic strain carrying the chr 1 modifier as homozygous B6 and the remainder of the genome as D2. In a cross with a B6 *jck/+* mouse, the congenic region on chr 1 is fixed as B6, while the rest of the genome is segregating as in an F2 population, and all the affected mice should be informative for the interacting D2 locus. Consistent with this hypothesis, we were readily able to localize on proximal chr 4 a D2 locus significantly associated with severe PKD. This localization is of particular interest because this is the position of a locus previously shown to modify the progression of PKD in two different mutations, *cpk* and *pcy*. This suggests this gene might influence PKD severity irrespective of its cause, and, as such, represents a potential target for therapeutic intervention in human PKD.

Program Nr: 508 from the 1999 ASHG Annual Meeting

Localization of Paroxysmal Kinesigenic Dyskinesia (PKD) to chromosome 16p11.2-q11.2. *L.B. Bennett[1], E.S. Roach[2], A.M. Bowcock[1]*. 1) Department of Genetics and Pediatrics, Washington University School of Medicine, St Louis, MO; 2) Dept Neurology, UT Southwestern Med Ctr, Dallas, TX.

Paroxysmal kinesigenic dyskinesia (PKD) is an autosomal dominant disorder characterized by episodic choreiform or dystonic movements brought on or exacerbated by voluntary movement or by the contemplation of voluntary movement. The abnormal movements usually begin between 6 and 16 years, and about half of the affected individuals have a family history. The biochemical defect responsible for the disease is not known. Neither is the genetic location of susceptibility alleles, the penetrance of disease alleles nor the extent of genetic heterogeneity. Most cases of PKD have been classified as familial although a smaller number have been described as acquired or symptomatic and have been associated with other neurological diseases such as multiple sclerosis or cerebral palsy.

We identified a large African-American kindred with the disease and performed a genome-wide scan with highly polymorphic microsatellites at 25 cM resolution. Linkage analyses localized the gene in this family to chromosome 16p11.2-q11.2. When a penetrance estimate of 80% was used a maximum two point LOD score of 3.76 at recombination fraction theta = 0.001 was obtained with D16S419. Critical meiotic-recombination events in one individual localize the disease gene in this family to a 20cM region between D16S3100 and D16S771. The non-kinesigenic form of the disease has been linked to chromosome 2q reinforcing the conjecture that the two diseases are distinct entities.

A gene for infantile convulsions with paroxysmal dyskinesia (ICCA syndrome) has also been mapped to Chr 16p12-q12 in families from France and China. The PKD and ICCA regions overlap by approximately 6cM. It is therefore tempting to speculate that these two diseases are due to different mutations in the same gene. Alternatively, chr 16p11-q11 may harbor several loci predisposing to familial convulsions.

Program Nr: 509 from the 1999 ASHG Annual Meeting

High-throughput SNP genotyping using single-base extension. *S. Bolk, J.N. Hirschhorn, N. Kalyanaraman, B.W. Langhorst, J.S. Ireland, P. Sklar, E.S. Lander.* Center for Genome Research, Whitehead Institute/MIT, Cambridge, MA.

With the identification of single nucleotide polymorphisms (SNPs) in the coding regions of hundreds of genes, there is a need for rapid, accurate and robust methods for genotyping SNPs. We are developing a spectrum of genotyping assays for use in association studies of complex diseases, mapping, and other applications. To genotype individual SNPs, we use single base extension (SBE) followed by fluorescence resonance energy transfer (FRET). In this technique, a FAM-labeled primer is extended by a single base in the presence of ROX and TAMRA-labeled ddNTPs. We have transformed this method into a high throughput platform that we have applied to ongoing large-scale association studies.

In order to develop SBE-FRET as a technique for cost-efficient, high-throughput genotyping, we have optimized the methodology as originally described by Chen et al. (PNAS 94, 1997). With the goal of optimizing genotyping quality across a large number of assays while minimizing cost, we have developed a set of optimal conditions for SBE-FRET. We evaluated a number of critical parameters including post-PCR clean-up and SBE reaction conditions. Our experience with optimized SBE-FRET genotyping has proven that this method is suitable for high-throughput SNP genotyping. SBE-FRET provides accurate, reproducible genotypes. In a pilot study of 30 loci to be used in SBE-FRET genotyping, we observed >99% reproducibility and >99% accuracy. SBE-FRET is generalizable to many polymorphisms; we have rapidly developed genotyping assays for over 180 cSNPs. Finally, SBE-FRET allows high-throughput genotyping and automated scoring of genotypes. We can now process 2,000 genotypes/day on one real-time PCR machine and have automated allele calling. We have begun association studies for bipolar disorder, coronary artery disease, and type II diabetes using SBE-FRET. To date, we have produced over 60,000 genotypes in these studies. We are also developing technologies designed to eliminate the need for locus-specific FAM-labeled primers, including the use of tagged SBE primers and a generic FAM-labeled oligonucleotide.

Program Nr: 510 from the 1999 ASHG Annual Meeting

b**-2 Adrenergic Receptor Gene Variations, Blood Pressure, and Heart Size In Normal Twins.** *A. Busjahn[1], G.-H. Li[2], H.-D. Faulhaber[1], H. Schuster[1], B. Timmermann[2], M.R. Hoehe[2], F.C. Luft[1].* 1) Franz Volhard Clinic, Berlin, Germany; 2) Max Delbrueck Center for Molecular Medicine, Berlin, Germany.

Genetic variability which influences cardiovascular phenotypes in normal persons is likely to be relevant to cardiovascular disease. We studied normal monozygotic and dizygotic twins and found strong genetic influences on blood pressure and heart size. We then relied on the dizygotic twins and their parents to apply molecular genetic techniques.

We performed a linkage analysis with markers close to the b-2 adrenergic receptor gene locus in the dizygotic twins and their parents, and found strong evidence for linkage to the quantitative traits blood pressure and heart size. We used allele-specific PCR to genotype the subjects further. We then performed a maximum likelihood association analysis based on sib pairs, and found that four functionally-relevant polymorphisms in the b-2 AR gene, namely Arg16/Gly, Gln27/Glu, Thr164/Ile, and a variant in the promoter region (-47C/T) were variably associated with blood pressure and heart size differences, but were in linkage dysequilibrium with each other. A subsequent conditional analysis suggested that the Arg16/Gly polymorphism exerted the predominant effect. Arg16 wildtype subjects had recumbent, sitting, and standing systolic blood pressures 7-8 mm Hg higher than homozygous Gly16 subjects, while heterozygous subjects were intermediate. Gly16 was also associated with a lesser cardiac septum thickness and lower norepinephrine levels. Additional interactions between the polymorphisms may be elucidated by larger studies with complex haplotype analyses.

These findings underscore the importance of the b-2 AR gene to blood pressure regulation, heart size, and probably also to the development of hypertension. We suggest that a combined linkage and association approach will elucidate the genetic variabilty influencing blood pressure and other cardiovascular phenotypes.

Program Nr: 511 from the 1999 ASHG Annual Meeting

Linkage analysis of 50 candidate genes involved in essential hypertension in 95 Chinese nuclear families with 460 affected sib-pairs. *S. Chu[1,2], M. Xiong[2], D. Zhu[1], G. Wang[1], Y. Zhan[1], W. Zhang[1], H. Zhou[1], H. Wang[1], W. Li[2], D. Shen[2], L. Jin[2,3]*. 1) Shanghai Institute of Hypertension, Ruijin Hospital, Shanghai Second Medical University, Shanghai, China; 2) Human Genetics Center, University of Texas Houston, Houston, TX; 3) National Human Genome Center at Shanghai, Shanghai, China.

Essential hypertension (EH), defined as elevated blood pressure (BP) due to unknown etiology, is a common and important risk factor for stroke, coronary heart disease, congestive heart failure, end-stage renal disease, and peripheral arterial disease. The complex nature of blood pressure determination is largely due to the fact that many genetic and environmental factors are involved and they may interact with each other in unpredictable ways. We investigated the linkage of EH, in 95 Chinese nuclear families containing 460 affected sib-pairs, at 45 chromosomal regions containing 50 candidate genes that have been implicated to the etiology of EH based on previous physiological, biochemical, and genetic studies. The distance between the candidate genes and the microsatellites selected ranges from 2 8cM. The two-locus, non-parametric linkage analysis (NPL) with GENEHUNTER indicated a positive linkage at D12S398 ($p = 0.021$). However, the application of the transmission/disequilibrium test (TDT) supports significant linkages, with small p values, at D8S261, D11S1347, D12S398, and D14S61, implicating LPL, APOA1-C3, V1AR, TGF-B3, respectively.

Program Nr: 512 from the 1999 ASHG Annual Meeting

All over the map: An integrated physical and genetic map of single nucleotide polymorphisms. *R.J. Clifford, M.N. Edmonson, T. Scherpbier, Y. Hu, C. Nguyen, A.K. Voltz, K.H. Buetow.* LPG/DCEG/NCI, NIH, Bethesda, MD.

The mission of the National Cancer Institute's Cancer Genome Anatomy Project is to provide a comprehensive description of the molecular changes underlying cancer initiation and progression. As part of the CGAP Genetic Annotation Initiative (CGAP-GAI), the Laboratory of Population Genetics is developing an extensive set of human single nucleotide polymorphisms (SNPs) as a resource for the molecular and epidemiological investigation of cancer. SNPs are predicted from publicly available EST sequences using computer algorithms, validated in the laboratory, then confirmed to segregate in a Mendelian fashion in CEPH families. Confirmed SNPs are positioned on the genetic map relative to the ABI version 1 marker panel. Our present collection of 10,243 candidate SNPs is derived from 6459 UniGene EST assemblies, 1967 of which correspond to named genes. 6485 SNPs have been placed on the genetic and/or physical maps. Information about CGAP-GAI SNPs is available online at (http://cgap.nci.nih.gov/GAI). Our website includes: i) an integrated genetic and physical map showing the locations of candidate, validated and confirmed SNPs, ii) a Java-based SNP viewer that displays polymorphisms in the context of EST assemblies, iii) a search engine that allows SNPs to be retrieved by keyword, gene symbol, or GenBank accession number, iv) genetic mapping reports for confirmed SNPs and v) access to our SNP prediction tools. Our site also contains links to the NCBI's UniGene and Genemap'98 sites.

Program Nr: 513 from the 1999 ASHG Annual Meeting

The Absent Granular Layer Histology in Ichthyosis Vulgaris is Linked to the Epidermal Differentiation Complex on Chromosome 1. *J.G. Compton[1], J.J. DiGiovanna[2], K. Johnston[3], P. Fleckman[4], S.J. Bale[1].* 1) National Institute of Arthritis, Musculoskeletal and Skin Diseases, NIH, Bethesda, MD; 2) Department of Dermatology, Brown University, Providence, RI; 3) Dermatology Service, 1st Medical Group, Langley AFB, VA; 4) Department of Medicine (Dermatology), University of Washington, Seattle, WA.

Ichthyosis vulgaris (IV) is a mild to severe scaling disorder of uncertain etiology estimated to affect as many as 1:250 in the population. Family studies have shown that in many cases IV follows an autosomal dominant inheritance pattern. To investigate the genetic basis for inherited IV, we have performed gene linkage studies in two multigeneration families with clinical IV but distinct histological features. The epidermis in this disorder characteristically displays non-specific orthohyperkeratosis. Reduced or absent granular epidermal layers and decreased filaggrin levels have been described. A subset of individuals has been characterized with the clinical findings of IV and a total absence of the granular layer. Because of the prominent role of profilaggrin in human keratohyalin comprising the granules, the absent granular layer pathology (AGL) has led to suggestions that defects in the gene for profilaggrin (FLG), its processing to filaggrin, or a gene involved in profilaggrin regulation may underlie IV. Family 1 had 7 individuals with IV, severe heat intolerance and epidermis with 1-3 granular layers (consistent with normal epidermal histology). IV in this family did not segregate with FLG or other genes in the epidermal differentiation complex. In contrast, five of the six IV patients in Family 2, all siblings, had epidermis with no granular layer. Significant evidence was obtained for linkage of the AGL phenotype, though not IV, to the epidermal differentiation complex (and FLG) assuming either a recessive (max Lod 3.4) or dominant (max Lod 3.6) inheritance model. Sequence analysis of FLG did not uncover a mutation in the amino or carboxyl terminal portions of the coding sequence adjacent to filaggrin repeats.

Program Nr: 514 from the 1999 ASHG Annual Meeting

Genetic mapping of familial hypertrophic-restrictive cardiomyopathy. *M.W. Consevage[1], S. Kasarda[1], D.W. Sabol[2], P.K. Rogan[3]*. 1) Division of Pediatric Cardiology, Penn State Univ Coll Medicine, Hershey, PA; 2) Phylogenetix Laboratories, Pittsburgh, PA; 3) Section of Medical Genetics and Molecular Medicine, The Children's Mercy Hospital and Clinics, Kansas City, MO.

Familial hypertrophic cardiomyopathy (HCM) is a genetically heterogeneous, autosomal dominant disorder that results from abnormalities of several sarcomeric force-generating proteins. Myocyte disarray and variable expressivity in the pattern and the degree of ventricular hypertrophy are characteristic of HCM. While mutations in the genes encoding these proteins account for ~50 - 60% of all HCM cases, the genetic etiology in the remaining patients has remained elusive. We have localized a previously undescribed locus for HCM in a clinically distinctive pedigree. Most affected members of this family demonstrate the usual finding of a non-obstructive HCM. However, two individuals, who initially had finding of a HCM phenotype, later progressed to restrictive cardiomyopathy (RCM). Similar RCM subtypes have been reported within FHCM families, but these genes responsible have not previously been mapped (Am J Cardiol. 80:1046-50). This familial HCM-RCM phenotype maps to a previously undescribed locus. Linkage to known HCM loci and 23 other sarcomeric loci was excluded for this family. A genome search demonstrated linkage to chromosome 5q33. Haplotype analysis has limited the disease interval to 6 cM, which includes the positional candidate gene encoding d-sarcoglycan. HCM has been reported in various muscular dystrophies (Int J Cardiol 16:205-8; Am J Med Sci 305:166-70; Am Heart J 128:1264-6), consistent with the possibility that particular mutations within these genes responsible for these disorder may also cause HCM. The Syrian cardiomyopathic hamster (BIO14.6), a widely used animal model for human HCM, results from mutations within the d-sarcoglycan gene(Hum Mol Genet 6:601-7), i.e., the same gene responsible for human limb-girdle muscular dystrophy type 2F (Nat Genet 14:195-8). These observations support the concept that an alternative set of genes related to the muscular dystrophies should be considered as candidates for HCM.

Program Nr: 515 from the 1999 ASHG Annual Meeting

HLA-DRB1*01 alleles are associated with skin test reactivity to cockroach allergens in Caucasians and African Americans. *J. Donfack[1], D. Hoki[1], R. Parry[2], L. Lester[3], C. Ober[1]*. 1) Departments of Human Genetics, University of Chicago, Chicago, IL; 2) Medicine, University of Chicago, Chicago, IL; 3) Pediatrics, University of South Dakota, Sioux Falls, SD.

A genome-wide search for loci that confer susceptibility to atopy was completed in the Hutterites, a founder population of European descent (Ober et al., 1999, *Clin Exp Immuno*, in press). Skin prick tests (SPT) to 14 common allergens were used as a measure of atopy. A positive (+) SPT results from previous exposure and a specific IgE response to the allergen. The marker locus, DQ.CAR, was significantly associated with +SPT to cockroach allergens using the TDT (P = 0.00001). DQ.CAR is located in the HLA class II region, making HLA class II loci excellent positional candidate genes for the DQ.CAR associated atopy-susceptibility locus. *HLA-DRB1*, *HLA-DQA1*, and *HLA-DQB1* genotypes were examined in 750 Hutterites with skin test phenotypes. The most significant associations were found between +SPT to cockroach allergens and *DRB1*0101* (P = 0.0006), *DQA1*0101* (P = 0.0002), and *DQB1*0501* (P = 0.0001). These alleles occur together on six haplotypes in the Hutterites, making it impossible to determine their individual effects on the allergic phenotype in this population. To replicate these findings and evaluate the individual effects of these class II loci on cockroach allergies, we studied 42 unrelated African American subjects who were +SPT to cockroach allergens and 45 African American subjects who had -SPT to cockroach allergens. The only allele that was significantly increased in cases compared with controls was the *DRB1*0102* allele (Frequency *DRB1*0102* positive cases vs controls 0.131 and 0.011, respectively; P = 0.0012). Neither *DQA1*0101* nor *DQB1*0501* was increased in the +SPT subjects (P > 0.20). *DRB1*0101* and *DRB1*0102* alleles differ only by two amino acid sites. Although HLA class II loci have previously been associated with specific IgE, these data are the first to suggest that HLA class II alleles confer susceptibility to cockroach allergens in Caucasians and African Americans. (Supported by NIH grants HL49596, HL56399, and HD21244.).

Program Nr: 516 from the 1999 ASHG Annual Meeting

AN INTEGRATED GENETIC LINKAGE MAP WITH 1140 MARKERS CONSTRUCTED FROM FIVE F2 CROSSES OF AUTOIMMUNE DISEASE -PRONE AND -RESISTANT INBRED RAT STRAINS. *S. Dracheva[1], E. Remmers[1], P. Gulko[3], Y. Kawahito[4], R. Longman[1], J. Wang[1], J. Shepard[1], L. Ge[1], B. Joe[1], G. Cannon[2], M. Griffiths[2], R. Wilder[1]*. 1) ARB, NIAMS/NIH, Bethesda, MD; 2) Research Service Veterans Affairs Medical Center and Department of Medicine/Rheumatology, University of Utah, Salt Lake City, UT; 3) Columbia University College of Physicians and Surgeons, New York, NY; 4) Koyto Prefectural University of Medicine, Kyoto 602, Japan.

The rat (Rattus norvegicus) is an important experimental model for many human diseases including arthritis, diabetes and other autoimmune and chronic inflammatory diseases. The rat genetic linkage map, however, is less well developed in comparison with those of the mouse and human. Here, we present an integrated linkage map with 1140 markers. We constructed this map by genotyping 200 F2 progeny of five crosses: F344/NHsd x LEW/NHsd (674 markers), DA/Bkl x F344/NHsd (532 markers), BN/SsN x LEW/N (715 markers), DA/Bkl x BN/SsNHsd (194 markers) and DA/Bkl x ACI/SegHsd (246 markers). These inbred rat strains vary in susceptibility/resistance to multiple autoimmune diseases and are used extensively for many types of investigation. The rat integrated map was independently constructed with two different programs: MAPMAKER and MULTIMAP. The total length of the MULTIMAP rat map at LOD score 1 was 5.5% larger than that of the MAPMAKER (1885.5 cM vs. 1787.4 cM). The order of the markers, placed in unique map positions by the MULTIMAP program was the same as on the map constructed using the MAPMAKER program. Thus, integrated maps constructed by two different methods confirmed each other. The integrated map includes 360 loci mapped in three or more of the five crosses. The map contains 196 new SSLP markers developed by our group, as well as many SSLP markers developed by other groups. Inclusion of the markers associated with 237 genes permits map comparison among species. This integrated map should allow comparison of rat genetic maps from different groups and facilitate genetic studies of rat autoimmune and related disease models.

Program Nr: 517 from the 1999 ASHG Annual Meeting

High Throughput Scanning for Variations by Mismatch Repair Detection (MRD). *M. Faham, S. Baharloo, S. Tomitaka, J. DeYoung, P. Gajiwala, N. Freimer.* Psychiatry, Univ. of Calif San Francisco, San Francisco, CA.

Various techniques have been developed for high throughput genotyping using single nucleotide polymorphisms (SNPs) with the aim of identifying disease-susceptibility alleles by linkage disequilibrium analyses. Using a static map of common SNPs identified in the general population may prove insufficient to identify alleles of common disease. Rare as well as common variants in the general or patient population may need to be tested to identify variations involved in common disease. Flexible approaches that can identify variations in different patient populations are needed. Therefore we believe that developing technologies that are capable of large scale scanning of the genome to find the disease-causing variations directly is of paramount importance. Mismatch Repair Detection (MRD) is a method that utilizes bacterial mismatch repair system in vivo to detect variations. In order to detect variations in many fragments simultaneously, we have developed MRD from its original form that was described earlier. We changed the experimental procedures to make the method more robust and eliminate the need for intermolecular ligation. More importantly, we have constructed a strain that we call Mutation Sorter (MS). When transformed with a mixture of DNA fragments, MS will sort the fragments into two pools: the variant and the nonvariant pools. The problem of DNA variation detection is then reduced to identifying which fragments are in which pool. This task can be performed by multiple methods including running an acrylamide gel or microarray hybridization. We expect that thousands of fragments can be analyzed simultaneously. Results from the MRD procedure are robust and reproducible. We will present results of scanning more than 40 fragments simultaneously.

Program Nr: 518 from the 1999 ASHG Annual Meeting

Examination of multiple loci in late onset familial Alzheimer disease (AD). *M.E. Garcia[1], L.R. Bailey[1], J.L. Hall[1], W.K. Scott[2], A.M. Saunders[5], G.W. Small[3], A.D. Roses[4], M.A. Pericak-Vance[2], J.L. Haines[1], P.M. Conneally[6].* 1) Prog in Hum Gen, Vanderbilt Univ Med Ctr, Nashville, TN; 2) Center for Hum Gen, Duke Univ., Durham, NC; 3) Dept. Psychiatry, Univ. California, Los Angeles, CA; 4) Glaxo Wellcome R&D, RTP, NC; 5) Dept. Medicine, Duke Univ., Durham, NC; 6) Dept. Med. Mol. Genet., Indiana Univ., Indianapolis, IN.

Apolipoprotein E (APOE) is the only known susceptibility gene for late-onset AD. Our previous genomic screen identified four potential locations (on chrs. 4, 6, 12, and 20) for one or more AD genes with the strongest results on chr. 12 (Pericak-Vance et al., JAMA, 1997). Further analysis indicated that the chrs. 4 and 20 results were false positives (Garcia et al., ASHG abst. 1673, 1998). The chr. 12 locus has been confirmed but does not explain all the remaining genetic effect on AD. The results on chr. 6 have remained consistently positive (lods 1-2) for many markers across multiple datasets. Lod score analysis revealed two regions of possible linkage on chr. 6 near markers D6S1019 (54 cM from ptel; lod=1.37) and D6S391 (90 cM from ptel; lod=0.85). We evaluated the independence of the chr. 6 genetic effect with both APOE and the chr. 12 locus by using a two-trait-locus ASP analysis (TWOLOC) on a dataset of 116 families with 227 ASPs, choosing the most informative and significant markers in each region. The analysis considered interactions with one of the following: 1) a locus on chr. 12 near markers D12S398 and D12S1632 or 2) the APOE locus. The two-locus models offered strong evidence of linkage (D6S1019: General MLS=12.83; D6S391: General MLS=6.97) using APOE (single locus MLS=5.42) as the second locus. Little evidence of interaction between loci was found since the differences between the general, additive, or multiplicative models were < 1 lod unit. It is not clear if the two regions on chr. 6 represent one or two loci. It is interesting to note that D6S1019 is near the MHC, a region implicated by both association and linkage studies (Small et al., Neurob Aging,1991; Kehoe et al., HMG,1999). These data suggest that a potential locus (or loci) on chr. 6 acts independently of both APOE and the susceptibility locus on chr. 12.

Program Nr: 519 from the 1999 ASHG Annual Meeting

A genetic region on chromosome 16 may predispose to the development of Crohn's disease at an early age in Ashkenazi Jews. *B. Gulwani-Akolkar[1], P.N. Akolkar[1], M. Daly[3], C. Sabatti[4], X-Y. Lin[1], S.E. Danzi[1], R. Pergolizzi[1], I. Storch[1], N. Risch[4], S. Katz[1], J. Levine[2], J. Silver[1].* 1) North Shore University Hospital/NYU Medical Center, NY; 2) Long Island Jewish/Einstein College of Medicine, NY; 3) MIT Genome Center, Cambridge, MA; 4) Stanford University, Stanford, CA.

Although the etiology of Crohn's disease (CD) is unknown there is strong evidence that genetic factors play an important role in its development. Recent genome-wide screening studies have suggested that there may be susceptibility loci on chromosomes 12 and 16; however, several groups have been unable to replicate these findings. To determine whether these loci play a role in CD in Ashkanazi Jews (AJ), 89 AJ affected-relative pairs were analyzed for linkage to the putative susceptibility loci on chr 12 and 16. In addition, 42 non-Jewish affected- relative pairs were analyzed for comparison. We observed weak linkage to the candidate region of chr 16 in the Ashkenazi population (NPL 1.53, p=0.06) and no linkage in the non-Jewish population. However, when the AJ population was subdivided on the basis of age-of-onset of CD there was a striking increase in linkage in the group where both affecteds within a family had an age-of-onset <21; this group displayed strong linkage to the chr 16 region (NPL 2.63, p=0.006). No similar increase in linkage was observed in the non-Jewish group where the age-of-onset was <21. When linkage to the chr 12 region was examined we observed evidence for linkage in the AJ population (NPL 2.09, p=0.02) but not in the non-Jewish population. However, in contrast to what was observed for chr 16, no increased linkage evidence was observed in the AJ group where the age-of-onset was <21. These data suggest that Ashkenazi Jews show increased linkage evidence on chromosomes 12 and 16. Furthermore, the striking increase in linkage in the <21 group suggests that the chr 16 region may predispose to the development of CD at an early age in the Ashkenazic Jewish population. This work was supported by funds from the New York Crohn's Foundation, Reach Out for Youth with Ileitis and Colitis, and a grant from the NIH.

Program Nr: 520 from the 1999 ASHG Annual Meeting

Detection of Modifier Loci Influencing the Lung Phenotype of Cystic Fibrosis Knockout Mice. *C. Haston, C. McKerkie, B. Budisin, T. Samanta, G. Kent, M. Corey, L-C. Tsui, R. Rozmahel.* Genetics, Hospital for Sick Children, Toronto, Ontario, Canada.

The majority of cystic fibrosis patients develop pulmonary disease and the severity of this complication appears to be independent of CFTR genotype. In this work mouse models of CF were used to investigate the hypothesis that CF associated lung disease is influenced, in part, by modifier genes. In order to identify the genetic factor(s) associated with predisposition of lung disease, a genome wide scan for quantitative trait loci (QTL) contributing to the observed differences in CF-associated lung disease between B6 and BALB Cftr-/- mice has been initiated. The pulmonary phenotype was assessed with three assays: a semi quantitative histological method based on the extent and severity of left lung pathology; image analysis of nucleic area per lung tissue area in a histological section, a quantitative measure of tissue cellularity; and myeloperoxidase levels in the right lung as an index of neutrophil influx. Histological results showed that B6 Cftr-/- mice had increased interstitial mononuclear cell infiltration with alveolar thickening and collagen deposition, relative to BALB Cftr-/- mice or control mice. The lung tissue cellularity of B6 Cftr-/- mice significantly exceeded that of BALB Cftr-/- mice and normal littermate controls (nuclei percent of 22.5±1.5 (SEM), 16.1±1.2, [p=0.011], and 13.5±0.9 respectively, for the three groups of animals). The right lung myeloperoxidase of BALB Cftr-/- mice exceeded that of B6 Cftr-/- mice, (141±35 units/gram of protein versus 55±21, p=0.028). Preliminary data have been obtained for 100 DNA markers, at 20 cM spacing, in 93 twelve week old F2 mice. The QTL analysis revealed one locus meeting the criterion of suggestive linkage for the alveolar thickening phenotype and a second suggestive locus was identified based on tissue cellularity. In conclusion, the distinct CF lung phenotypes in two congenic CFTR-knockout mouse strains should permit the identification of the modifier genes.

Program Nr: 521 from the 1999 ASHG Annual Meeting

Affected sibpair analysis: Exploring the efficiency of fine mapping in the presence of genotyping error. *E.R. Hauser[1], A. Ashley-Koch[1], M.A. Pericak-Vance[1], M. Boehnke[2].* 1) Dept of Medicine, Duke Univ Medical Ctr, Durham, NC; 2) Dept of Biostatistics, Univ of Michigan, Ann Arbor, MI.

We examined the impact of genotyping error on the efficiency of fine mapping data to localize a gene to a small interval. Feakes et al (1999), using empirical data from a multiple sclerosis genomic screen, suggested the limit of efficiency in fine mapping of complex disorders is a 2.5 to 5 cM map. Past that point, loss of information from map and genotype errors (including mutation) outweighed the information gained from increased marker density. We explored the problem of genotype error and map density by simulating SNP and microsatellite data for affected sibpairs. We used a chromosome of 100 cM in length, with a disease locus at 52 cM. Two or four equally frequent alleles were simulated for each SNP or microsatellite marker, respectively. We assumed markers were in correct map order and all families were linked to the disease locus. Using an additive model, we considered sibling recurrence risks (l_s) of 1.0, 1.4, 1.6 and 2.0. Genotype error rates were set at 0%, 0.1%, 0.5%, 1.5%, 3% or 5%. Marker densities of 2, 5, 10 and 20 cM were evaluated. For each analysis, 1000 replicates of 100 or 400 families were simulated. For high error rates (3-5%), useful information can be extracted by increasing map density to 2 cM if the sibling recurrence risk is relatively large ($l_s \geq 1.6$). However, if the recurrence risk is small ($l_s < 1.6$), there is little utility in increasing map density past 5-10 cM. For low error rates ($\leq$1.5%), useful information is still extracted with a 2 cM map, regardless of l_s. We also examined the effect of genotyping error on the estimate of the disease gene location. Our results suggest *random* genotyping error does not produce bias in the estimate, but does increase the variability of the estimate. We are continuing to examine effects of high error rates for individual markers and marker order misspecification. In conclusion, we agree with Feakes et al (1999) that genotyping error can decrease the efficiency of your mapping endeavor. However, with low genotyping error rates and accurate marker order, useful information can still be obtained with dense maps.

Program Nr: 522 from the 1999 ASHG Annual Meeting

Mapping a locus for lymphedema-distichiasis to chromosome 16q24.3. *S. Jeffery[1], J. Mangion[2], N. Rahman[2], S. Mansour[1], G. Brice[5], J. Rossbotham[5], A.H. Child[5], A.L. Evans[5], P. Mortimer[5], R. Barfoot[2], A. Sigurdsson[2], S. Edkins[2], M. Sarfarazi[4], K. Burnand[3], T. Nunan[3], M.R. Stratton[2], V.A. Murday[1].* 1) Medical Genetics, St. George's Medical School, London, England; 2) Institute of Cancer Research, London, England; 3) St Thomas' Hospital, UMDS, London, England; 4) Surgical Research Center, Dept of Surgery, University of Connecticut; 5) Cardiology and Dermatology, St George's Medical School, London,.

Lymphedema-distichiasis (LD) is a dominantly inherited syndrome with onset of lymphedema at or just after puberty. Most affected individuals have distichiasis, fine hairs arising inappropriately from the eyelid meibomian glands, evident from birth. We have analysed three families with this disorder, and found a locus for LD on chromosome 16q24.3, the first localisation for this form of lymphedema. Subsequent analysis of the region for recombinants places the locus between D16S422 and D16S3074, a distance of about 16cM. Possible candidate genes in this interval include the N-proteinase for type 3 collagen PCOLN3, the metalloprotease PRSM1 and the cell matrix adhesion regulator CMAR.

Program Nr: 523 from the 1999 ASHG Annual Meeting

Strategies for fine mapping of QTLs contributing to variation in peak bone density. *D.L. Koller[1], M.J. Econs[1], P.A. Morin[2], J.C. Christian[1], S.L. Hui[1], P. Parry[2], M.E. Curran[2], L.A. Rodriguez[2], P.M. Conneally[1], G. Joslyn[2], M. Peacock[1], C.C. Johnston, Jr.[1], T. Foroud[1]*. 1) Indiana Univ. School of Medicine, Indianapolis, IN; 2) Axys Pharmaceuticals Inc., La Jolla, CA.

Osteoporosis is a leading public health problem that is responsible for substantial morbidity and mortality. A major determinant of the risk for osteoporosis in later life is peak bone mineral density (BMD) attained during early adulthood, which is largely determined by genetic factors. We conducted an autosomal genome screen for loci underlying BMD variation in 429 Caucasian sister pairs, computing LOD scores for BMD at four skeletal sites using the maximum likelihood variance method in Mapmaker/SIBS. LOD scores above 1.85 were further pursued in an expanded sample of 595 sister pairs. The highest LOD score attained in the expanded sample was 3.86, at chromosome 1q21-23 with lumbar spine BMD. Chromosomes 5q33-35 (LOD=2.23), 6p11-12 (LOD=2.13) and 11q12-13 (LOD=2.16) also produced interesting linkage findings. We employed non-parametric linkage analysis in sibships, as is typical in genetic studies of complex disease, and observed several instances where particular individuals or sibships exerted a disproportionate influence on the linkage results. Siblings with extreme phenotypic values produced large differences in LOD score results when included or excluded, as did single half-sibling pairs included as full sibs prior to detection of their true relationship. Variable informativeness among nearby markers, especially in one five-sibling pedigree without genotyped parents, led to highly discrepant two-point and multipoint linkage results at the same marker position. Our study is the largest genome screen to date for genes underlying variation in peak BMD, and represents an important step toward identifying genes contributing to osteoporosis in the general population. While genome-wide linkage analyses are a necessary initial step toward this goal, detailed analysis of the contribution of individual sibships and families to linkage results will likely yield important insights necessary for successful fine-mapping and eventual localization of genes for complex disorders.

Program Nr: 524 from the 1999 ASHG Annual Meeting

Mapping of the locus for autosomal dominant renal Fanconi Syndrome. *U. Lichter-Konecki[1], K.W. Broman[1], R. Dart[2], E. Blau[2], D.S. Konecki[1].* 1) Center for Medical Genetics, Center for Medical Genetics, Marshfield Ctr Medical Genetics,Marshfield Medical Res Fndn, Marshfield, WI, USA; 2) Marshfield Clinic, Marshfield, WI, USA.

Familial renal Fanconi Syndrome is a genetic model for the study of the pathophysiology of renal tubular transport. Affected individuals are normal at birth. However, during the second decade of life they develop polyuria, and loss of proximal tubular function results in rickets. With increasing age, these individuals may develop renal failure. Therapy for renal Fanconi Syndrome consists of symptomatic supplementation therapy, dialysis, and kidney transplantation. The isolation of the affected gene should initially facilitate postnatal and prenatal diagnosis of renal Fanconi Syndrome. Long term, the isolation of this gene and its gene product will allow the study of the pathophysiology of renal Fanconi Syndrome. Such studies should lead to a better understanding and consequently a better treatment of this entity. Through knowledge about the pathophysiology of this disorder new drugs may be developed to treat renal tubular transport disorders. We were able to map the locus for autosomal dominant familial renal Fanconi Syndrome to the long arm of human chromosome 15 by genotyping a large Central Wisconsin pedigree by performing a whole genome scan with 367 highly polymorphic simple sequence repeat (SSR) markers. A maximum LOD score of 3.01 was calculated using the LINKAGE program package (version 5.03), with the analyses conducted assuming a fully penetrant autosomal dominant mode of inheritance. For the linkage analysis, younger individuals were treated as possessing unknown phenotype. The fine mapping of the locus for autosomal dominant renal Fanconi Syndrome is currently in progress, the goal of which is to sufficiently narrow the region to allow the isolation of the associated gene through a positional cloning approach.

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Program Nr: 525 from the 1999 ASHG Annual Meeting

Mitochondrial DNA polymorphism detection and heteroplasmy quantitation using high performance liquid chromatography. *M. Marino[1], J. Devaney[1], B. Levin[2], L. Tully[2]*. 1) Transgenomic, Inc., Gaithersburg, MD; 2) National Institute of Standards and Technologies, Biotechnology Division, Gaithersburg, MD.

Sequence analysis confirms the presence or absence of single nucleotide polymorphisms along with an abundance of additional sequence information. Minimizing time, expense, and data acquisition are critical concerns of large-scale initiatives. To evaluate suspected polymorphic sites alternative technologies are desired. A new chromatographic technique, denaturing high performance liquid chromatography (DHPLC) separates the DNA fragments based on their size, sequence, and charge density. Using DHPLC to screen the samples for heteroduplex formation reduces analysis time compared to sequencing. In this study, we investigate DHPLC for determination of polymorphisms of the mtDNA genome. The control region of the mitochondrial genome contains highly polymorphic areas of DNA sequence referred to as hypervariable regions. These unique sequences are maternally inherited and exist in high copy number, which enhance their use for forensic and genealogical determinations. Optimal elution temperature (59°C) and gradient conditions (1% increase in acetonitrile/minute) for analysis of the 440 bp fragment of the HV 1 region (15970F / 16410R) were determined and applied to various polymorphic pairs. Individuals with one single nucleotide polymorphism, compared to the Anderson (i.e. reference) sequence at positions 16067 (T ® C) and 16343 (A ® G) within the PCR-amplified fragment were evaluated for heteroduplex detection. To evaluate heteroplasmy of the mitochondrial genome, a silent mutation within the coding region was used to simulate heteroplasmy at various ratios of wild type to mutant. Individuals with the wild-type (C) and mutation (T) nucleotide at position 6371 were PCR-amplified and the concentration of DNA in each PCR product was determined by DHPLC. This quantitation allowed the products to be combined in different concentrations to determine the limits of heteroplasmy detection. These heteroplasmic products will be the basis of a Standard Reference Material being developed at the National Institute of Standards and Technology.

Program Nr: 526 from the 1999 ASHG Annual Meeting

Genetics of lupus: fine mapping of the Fc g **receptor gene cluster at chromosome 1q22-23.** *K.L. Moser[1], J.C. Edberg[2], J. Kelly[1], C. Gray-McGuire[1], N. Asundi[1], H. Yu[1], Y. Zhu[2], K. Chen[2], B.R. Neas[1,3], J.E. Salmon[4], R.P. Kimberly[2], J.B. Harley[1,3,5]*. 1) Oklahoma Medical Research Foundation, Oklahoma City, OK; 2) Univ. of Alabama at Birmingham, Birmingham, AL; 3) Univ. of Oklahoma, Okla. City, OK; 4) Hospital for Special Surgery, NY, NY; 5) VA Medical Center, Okla. City, OK.

Systemic lupus erythematosus (SLE) is a genetically complex autoimmune disease of unknown origin. Deficiency in Fcg receptor (FcgR) function was observed in SLE patients over 20 years ago. Significant associations have been found with functionally important allelic polymorphisms in several of the FcgR genes. More recently, a genome scan of 94 multiplex SLE pedigrees suggested linkage at the low affinity FcgR gene cluster at chromosome 1q22-23. We have now performed fine mapping in this region by genotyping additional microsatellites and alleles of the FcgRIIa, FcgRIIIa and FcgRIIIb candidate genes in 40 African-American (AA), 77 European-American (EA), and 9 other multiplex SLE pedigrees (total n=126). Data were analyzed using the model-based two-point lod score, sib-pair, and Transmission Disequilibrium Test (TDT) methods implemented in the ANALYZE package. Lod scores of 3.97 and 3.43 were found for the FcgRIIa and D1S2762 loci, respectively, using a recessive model with 50% penetrance. Supportive evidence for linkage was obtained from surrounding loci using both lod score (lods>1.5) and sib-pair analyses (0.05>p>0.0001). Analysis by TDT identified an effect between SLE and alleles at the FcgRIIIa locus (p = 0.0002) but not at the FcgRIIa locus (p=0.15) or the FcgRIIIb locus (p=0.50). Unlike earlier analyses of FcgRIIa that suggested significant differences in the contribution of FcgRIIa between AA and EA pedigrees, preferential transmission of the associated FcgRIIIa allele from parents to affected offspring was found in both groups (p=0.006, AA; p=0.002, EA). Analysis by TDT in each ethnic group was not significant for FcgRIIa (p=0.08, AA; p=0.5, EA) or FcgRIIIb (p=0.05, AA; p=0.5, EA). Given the known functional differences between FcgR alleles, these data indicate that FcgRIIIa is a strong candidate gene for susceptibility to SLE.

Program Nr: 527 from the 1999 ASHG Annual Meeting

Linked modifier at the Huntington's disease locus. *R.H. Myers[1], A. Novelletto[2], L.A. Cupples[3], A. DeStefano[1,3], J. Srinidhi[4], I. Kornbluth[1], J.F. Gusella[4], M.E. MacDonald[4].* 1) Boston Univ Sch Medicine, Boston, MA; 2) Universit Tor Vergata, Rome, Italy; 3) Boston Univ Sch Public Health, Boston, MA; 4) Massachusetts General Hospital, Harvard Med Sch, Boston, MA.

Huntington's disease (HD) has a mean onset age of 40years, but onset is highly variable. HD is attributable to a triplet CAG repeat on chromosome 4p16.3, which accounts for about 50% of the variation in onset age among persons with mid-life onset. In an affected sib-pair design, we sought to determine whether the size of the normal repeat at the HD locus influences disease expression and whether there is evidence for a linked modifier on the normal chromosome. Sibships with at least two affected siblings were collected from Italian (n=88 sibs) and New England (n=64 sibs) families. The Italian sample had a smaller HD repeat size (p=0.004), smaller normal repeat size (p=0.04) and later onset age (p=0.01); Italian sample: HD allele mean=43.6, normal allele mean=17.9, mean onset age=44.1 years, New England: HD allele mean=46.5, normal allele mean=19.1, mean onset afe=38.6 years. Onset age was adjusted for HD repeat size modeled as [log(onset)= a+b(CAG)]. Some analyses adjusted for HD repeat size, and an interaction of the HD and normal allele sizes. Analyses controlled for correlated observations for siblings. The normal repeat size was not associated with the age at onset in analyses unadjusted for the HD repeat, but a significant association between the normal repeat and onset age was seen in the Italian sibs (p=0.03) and in the combined data (p=0.02) after adjustment for the HD allele. A significant interaction of HD and normal allele sizes with onset age was seen in both the Italian (p=0.002) and New England samples (p=0.05), after adjustment for the HD and normal alleles. Larger normal alleles were associated with later onset age. Using sib-pair linkage strategies, significant evidence for linkage of a modifier locus on the normal chromosome, other than the normal repeat itself, was found for onset age after adjustment for the HD repeat and normal repeat sizes (p=0.003). Similar normal chromosome haplotypes in the two samples were associated with younger onset ages.

Program Nr: 528 from the 1999 ASHG Annual Meeting

Correcting for population stratification in linkage disequilibrium mapping studies. *J.K. Pritchard, M. Stephens, P.J. Donnelly.* Dept Statistics, University of Oxford, Oxford, UK.

In linkage disequilibrium mapping, the presence of population structure can lead to spurious associations between a phenotype and unlinked candidate loci. One common approach to dealing with this problem is to use certain family-based study designs such as the TDT, which are not invalidated by population structure. Here, we present an alternative approach, using genetic information to correct for the presence of population structure in the context of case-control studies. We anticipate that our approach will be of particular value in situations where it is difficult to collect family-based controls, such as for late-onset diseases. Our approach is to use multi-locus genotype data from a case-control sample to infer the details of population structure in the sample. The problem with population structure arises because allele frequencies can vary across ethnic groups. We have developed a method, using bayesian clustering, which uses this variation in allele frequencies to detect the presence of population structure, and to assign individuals (probabilistically) to the appropriate subpopulations. It is not necessary to identify subpopulations before applying the technique. A modified test of association can then be performed, taking account of the inferred population structure. We present results illustrating the performance of this method when applied to real and simulated case-control data.

Program Nr: 529 from the 1999 ASHG Annual Meeting

Schizophrenia: The Identification of Genetic Subgroups. *A.E. Pulver[1], S.E. Antonarakis[3], J.L. Blouin[3], D. Housman[4], H. Kazazian[2], V.K. Lasseter[1], J.G. Mulle[1], G. Nestadt[1], P. Wolyniec[1].* 1) Johns Hopkins Univ Baltimore, MD; 2) Univ of Penn, Phila., PA; 3) Univ of Geneva, Geneva, Switzerland; 4) Mass Inst of Tech, Cambridge, Mass.

Schizophrenia is a common, complex phenotype with strong evidence of genetic susceptibility. Although there are no symptoms that are pathognomonic, inter-rater and test- retest reliability has been established for the diagnosis. Family studies have suggested that the relatives of schizophrenic probands may be at an increased risk for several psychiatric diagnoses i.e., schizophrenia, other psychotic disorders and schizophrenia spectrum personality disorder(schizoid, schizotypal and paranoid personality disorders [SSPD]). Genetic heterogeneity is likely. Previously our group reported the results of a genome scan of 54 families which had at least two relatives diagnosed with schizophrenia. Non-parametric linkage analysis provided significant evidence for a schizophrenia susceptibility locus (SSL) on chromosome 13q32 (score 4.18; p= 0.00002) and suggestive evidence for 8p21 (score 3.64; p=0.0001). Additional regions providing some evidence included 7q11, 14q13 and 22q11. Given the probable heterogeneity, we have now subdivided the 54 families based on whether or not any first or second degree relatives of the proband had been given a diagnosis (based on direct assessment) of a SSPD or psychotic affective disorder (PAD). Seven families had at least one relative with a diagnosis of SSPD and six families had at least one relative with a diagnosis of a PAD. Non- parametric linkage analyses of markers throughout the genome suggests these two subgroups of families may be associated with different SSL. The strongest evidence for a SSL for the families segregating both schizophrenia and SSPD is found for 8p21-22 (score 5.04; p=0.000001)and 1pcen-1p21 (score 2.72; p= 0.0062). The strongest evidence for a SSL for the families segregating both schizophrenia and PAD is found for 22q13 (score 2.94; p=0.0044)and 3p25-3p22 (score 2.71; p=0.008). The identification of genetically more homogenous subgroups of families will facilitate the identification of SSL.

Program Nr: 530 from the 1999 ASHG Annual Meeting

A novel nonsyndromic recessive form of deafness maps to 4q28 and demonstrates incomplete penetrance. *S. Riazuddin[1,2], C.M. Castelein[3], T.B. Friedman[1], A.K. Lalwani[3], N. Liburd[1], S. Naz[2], T.N. Smith[1], S. Riazuddin[2], E.R. Wilcox[1]*. 1) Laboratory of Molecular Genetics, NIDCD, 5 Research Ct., 2A-19, Rockville, MD 20850-3227; 2) Centre for Excellence in Molecular Biology, Canal Bank Road, Niaz Thokar Baig, Punjab University, New Campus, Lahore, Pakistan; 3) Department of Otolaryngology-Head and Neck Surgery, University of California, San Francisco, 533 Parnassus Ave., Rm U490A, San Francisco, CA 94143-0526.

Congenital nonsyndromic deafness is usually inherited as a simple Mendelian trait with extreme heterogeneity. We report here the localization of a novel recessive nonsyndromic deafness locus (DFNB26) on chromosome 4q28 segregating in a large consanguineous Pakistani family. Thirty-three members of this extended family have enrolled in our study, among which are seven deaf individuals from five different consanguineous marriages. The seven deaf family members with severe to profound hearing loss share identical genotypes at 5 different closely linked STRPs. A maximum LOD score of 6.22(q = 0) was observed at D4S1610 based on an affecteds-only calculation. Haplotype analysis defines a maximum interval of 1.5 cM for this locus. We are generating a radiation hybrid map and BAC contig of this region as we attempt to identify the responsible gene. Surprisingly, there are four individuals with normal hearing that are homozygous for the same haplotype as their affected siblings. We hypothesize that there is a modifier inherited by these four individuals, thus compensating for their inherited DFNB26 locus. Because of the large number of enrolled family members, we should be able to map and identify the modifier that allows four normal individuals to escape the deafness inherited by their siblings.

Program Nr: 531 from the 1999 ASHG Annual Meeting

An autosomal dominant thrombocytopenia gene maps to chromosomal region 10p. *A. Savoia[1], M.E. Del Vecchio[1], A. Totaro[1], S. Perrotta[2], G. Amendola[3], A. Moretti[4], L. Zelante[1], A. Iolascon[4].* 1) Medical Genetic Service, IRCCS Ospedale CSS, San Giovanni Rotondo, Foggia, Italy; 2) Department of Pediatrics, II University of Naples, Naples; 3) San Leonardo Hospital, Castellamare di Stabia, Naples; 4) Department of Biomedicine of Evolutive Age, University of Bari, Bari.

Inherited thrombocytopenias are rare and heterogeneous disorders with autosomal and X-linked traits. In the majority of cases, the molecular basis of the disease is unknown although, the defect is likely to affect thrombocytopoiesis and the regulation of the normal platelet count. We report a genome-wide search in a large Italian family with autosomal dominant thrombocytopenia. Patients had a moderate thrombocytopenia with minimal symptoms characterized by normocellular bone marrow, normal medium platelet volume, and positive aggregation tests. Under the assumption of complete genetic penetrance, marker D10S197 gave a maximum two-point LOD score value of 3.91 at recombination fraction (q) of .00. The two flanking microsatellites D10S548 and D10S208 showed a linkage to the hrombocytopenia locus (*THC2*) with a maximum LOD score of 4.96 and 3.57 at q of .07 and .09, respectively. The marker density was further increased with nine additional microsatellites. Markers D10S588 and D10S1771 yielded the maximum two-point LOD score values of 8.12 and 7.52 (q=0), respectively. A centromeric recombination was evident between D10S588 and D10S1639. Another crossing over between D10S586 and D10S572 established the distal limit of the disease-region at D10S572. Microsatellite analysis showed the disease locus linked to chromosome 10p11.1-12, within a candidate region of 6 cM between markers D10S586 and D19S1639 The localization of the *THC2* locus represents an important step in the understanding of the genetic basis of inherited thrombocytopenias. New families will be tested for linkage to the chromosome 10p. Pedigrees that are not compatible with this locus could then be used for genome-wide search and localization of other genes. The search of the gene through the candidate gene approach could add clues to the understanding, at the functional level, of the molecular defects causing the platelet reduction.

Program Nr: 532 from the 1999 ASHG Annual Meeting

The NCBI dbSNP database for Single Nucleotide Polymorphisms and other classes of minor genetic variation.
S.T. Sherry, M. Ward, K. Sirotkin. NCBI, NIH, Bethesda, MD.

A key aspect of research in genetics is associating variations with heritable phenotypes. The most common variations are single nucleotide polymorphisms (SNPs), which occur approximately once every 100 to 300 bases in a large sample of aligned human sequence. Because SNPs are expected to facilitate large-scale association genetics studies, there has recently been great interest in SNP discovery and detection. In collaboration with the National Human Genome Research Institute, the National Center for Biotechnology Information (NCBI) has established the dbSNP database (http://www.ncbi.nlm.nih.gov/SNP) to serve as a central repository for molecular variation.

Designed to serve as a general catalog of molecular variation to supplement GenBank. Database submissions can include a broad range of molecular polymorphisms: single base nucleotide substitutions, short deletion and insertion polymorphisms, microsatellite markers, and polymorphic insertion elements such as retrotransposons. Once described, these variations exist as a public resource for future research, as dbSNP entries include information on the flanking sequence surrounding the variation, the specific experimental conditions necessary to characterize the variation, and population genetic variation information as either population-specific allele frequencies or individual genotypes.

As with all NCBI projects, the data in dbSNP are freely available to the scientific community and are available in a variety of formats via FTP from the SNP homepage. Submission instructions and online queries are accessible via the dbSNP homepage.

Program Nr: 533 from the 1999 ASHG Annual Meeting

Minimization of the Mucolipidosis Type IV candidate region on human chromosome 19 by haplotype analysis and detailed physical mapping. *S.A. Slaugenhaupt[1], J.S. Acierno[1], L.A. Helbling[1], C. Bove[2], G. Bach[4], L. Ashworth[5], E. Goldin[3], R. Schiffmann[3].* 1) Harvard Institute of Human Genetics, Harvard Medical School, Boston, MA; 2) Molecular Neurogenetics Unit, Massachusetts General Hospital, Charlestown, MA; 3) Developmental and Metabolic Neurology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD; 4) Department of Human Genetics, Hadassah University Hospital, Jerusalem, Israel; 5) Human Genome Center, Lawrence Livermore National Laboratory, Livermore, CA.

Mucolipidosis Type IV (MLIV) (MIM 252650) belongs to a group of inherited metabolic diseases known as the lysosomal storage disorders. It was first described in 1974 as a new variant of the mucolipidoses and was characterized by corneal clouding and abnormal systemic storage bodies. The absence of mucopolysaccharide excretion and the lack of identifiable lysosomal enzyme changes distinguish it from the mucopolysaccharidoses and from the other mucolipidoses. MLIV is a progressive neurological disease that usually presents during the first year of life with mental retardation, corneal opacities, and delayed motor milestones. To date more than 80 MLIV patients have been reported, the majority belonging to the Ashkenazi Jewish (AJ) population.

Using 13 AJ families, we originally mapped the gene to a 5.62 cM region on chromosome 19p13.2-13.3. Detailed analysis of 65 MLIV chromosomes (33 families) now demonstrates that two founder haplotypes account for 95% of the disease chromosomes in the AJ population. Examination of ancestral recombinants has narrowed the candidate region to 1.2 cM. We have constructed a detailed BAC and cosmid map that spans 1.4 Mb and includes the entire MLIV candidate region. We have recently started gene identification using methods including direct sequence prediction, EST mapping, exon trapping and cDNA selection. Isolation of the MLIV defect will permit a genetic prenatal test, a carrier test for the AJ population, as well as a simple tool for diagnosis and differentiation from the other lysosomal storage disorders.

Program Nr: 534 from the 1999 ASHG Annual Meeting

Linkage disequilibrium mapping identifies a Class III Major Histocompatibility Complex (MHC) susceptibility haplotype to Crohn's disease in Ashkenazi Jews. *K.D. Taylor, H. Yang, T.D. Hang, Y-C. Lin, S.R. Targan, N. Fischel-Ghodsian, J.I. Rotter.* Division of Medical Genetics and Inflammatory Bowel Disease Center, Cedars-Sinai Medical Center, Los Angeles, CA.

Background: Crohn's disease (CD) is a complex genetic disease. Ashkenazi Jews have a 2-4 fold increased susceptibility to CD compared with non-Jews. We, along with others, have observed evidence for linkage of CD to the MHC in both Jews and non-Jewish Caucasians. **Aim:** To characterize this genetic susceptibility to CD further using a linkage disequilibrium mapping approach with both population-based and family-based study designs in this high risk ethnic group. **Methods:** The case control panel consisted of 108 CD and 69 controls. The family panel consisted of 56 families with at least one member affected with CD and none with ulcerative colitis (196 total). All subjects were Ashkenazi Jews. Both panels were typed with 21 MHC markers spaced 0.2-0.4 Mb. The association of markers with CD in the case control panel was tested by chi-square tests. The TDT was performed in the family panel with single markers individually as well as with 2-, 3-, and 4-marker haplotypes using Genehunter 2.0. **Results:** Notch4 allele 2 was associated with CD in both the case control (p=0.011) and family panels (31 transmitted to affecteds (T), 14 not transmitted (NT); p=0.011). A 2-2 haplotype formed with the adjacent SNP marker HSP-HOM yielded T 29, NT 11 (p=0.0044). Incorporation of the next marker in the series, D6S273, created a Notch4-->HSP-HOM-->D6S273 2-2-4 haplotype that yielded 16 T, 2 NT (p=0.00097). These 3 markers span 0.5 Mb within the MHC Class III. **Conclusions:** These observations strongly suggest that a genetic variation in linkage disequilibrium with this MHC Class III 2-2-4 haplotype contributes to susceptibility to CD in Ashkenazi Jews. The association reported here is the strongest that we have observed between MHC markers and CD in this population to date. When combined with other observed associations to CD in this region, these results raise the possibility that multiple genes in the MHC contribute to susceptibility to CD and illustrate the complexity of even one chromosomal region in a common disese.

Program Nr: 535 from the 1999 ASHG Annual Meeting

Familial Hypomagnesemia with Hypercalciuria and Nephrocalcinosis maps to chromosome 3q27. *S. Weber[1], K. Hoffmann[2], K. Saar[2], N. Jeck[1], M. Boeswald[1], E. Kuwertz-Broeking[1], I. Meij[1], N. Knoers[1], P. Cochat[1], T. Sulakova[1], M. Soergel[1], F. Manz[3], H.W. Seyberth[1], K. Schaerer[4], A. Reis[2], M. Konrad[1]*. 1) University Children's Hospital, Marburg, Germany; 2) Mikrosatellitenzentrum, Max-Delbrueck-Centre, Berlin, Germany; 3) Research Institute of Child Nutrition, Dortmund, Germany; 4) Department of Pediatric Nephrology, University Childrens Hospital, Heidelberg, Germany.

Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC, MIM 248250) associated with chronic renal failure is a complex renal tubular disorder characterized by hypomagnesemia, hypercalciuria, advanced nephrocalcinosis, polyuria, hyposthenuria, low citrate excretion and a low glomerular filtration rate. Recurrent urinary tract infections, nephrolithiasis, incomplete distal tubular acidosis and eye involvement have also been reported. The mode of inheritance is autosomal recessive. To date, the underlying genetic defect and pathophysiology are unknown but a primary defect in the reabsorption of divalent cations in the medullary thick ascending limb of the loop of Henle (mTAL) has been proposed. To identify the underlying genetic defect we performed linkage analyses in eight families, including three with consanguineous marriages. After exclusion by haplotype analyses of several candidate genes coding for proteins involved in tubular fluid and electrolyte transport and loci for dominant isolated renal magnesium loss and familial hypomagnesemia with hypocalcemia (11q23 and 9q12-22.2 respectively) we performed a whole genome scan and found linkage to microsatellite markers on chromosome 3q27. A maximum two-point LOD score (Zmax) of 5.208 was found for D3S3530 at a recombination fraction Q= 0.00, whereas multipoint analysis gave a Zmax of 7.368 around marker D3S2398. Haplotype analysis revealed crucial recombination events reducing the critical interval for this locus (*HOMG3*) to 6.6 cM between markers D3S1580 and D3S3663. The identification of *HOMG3* will not only provide insight into the pathophysiology of FHHNC but might also give important clues into mechanisms of renal magnesium and calcium handling.

Program Nr: 536 from the 1999 ASHG Annual Meeting

Power of Variance Component Linkage Analysis to Detect Quantitative Trait Loci. *J.T. Williams, J. Blangero.* Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX.

Variance component methods for quantitative trait linkage analysis are highly flexible and provide superior statistical properties in many situations. To assist investigators in designing or evaluating linkage studies we determined the sample size required to achieve any desired power in variance component linkage analysis of a quantitative trait. For simplicity we consider an additive model comprising effects due to a single QTL, residual additive genetic factors, and individual-specific environmental variation. Exact expressions for the required sample size are given for sibpairs, sib trios, nuclear families having two and three sibs, and for arbitrary relative pairs. For larger sampling structures such as extended pedigrees the relevant equations are solved numerically. The power of the variance component method is dominated by the QTL-specific heritability, which is itself a function of the QTL allele frequency and the displacement between homozygote means. Comparative power curves are presented for sibships of size 2-4, nuclear families having 2-4 sibs, and for an extended pedigree of 48 individuals. Our results indicate that power is increased when the QTL acts in the presence of nonzero residual additive genetic variance. Omission of parental information in sibpair analysis will result in relatively little loss of information only for traits with low heritabilities. The procedures are easily applied to other covariance models and pedigree structures.

Program Nr: 537 from the 1999 ASHG Annual Meeting

Genome-wide linkage scan for familial idiopathic scoliosis. *C.A. Wise[1], R. Barnes[2], J.A. Herring[1], J.D. Gillum[1], L. Macleod[1], H.H. Hobbs[2], A.M. Bowcock[3], M. Lovett[3].* 1) Research Dept, Texas Scottish Rite Hospital, Dallas, TX; 2) Center for Human Genetics, University of Texas Southwestern Medical Center, Dallas, TX; 3) Department of Genetics, Washington University School of Medicine, St. Louis, MO.

Idiopathic scoliosis (IS) is defined as lateral curvature of the spine of unknown etiology. IS in human populations has estimated occurrences of 1.5-3%, 0.3-0.5%, and 0.2-0.3% for curves over 10, 20, and 30 degrees, respectively. An apparent genetic basis for IS is well recognized clinically, and a multifactorial inheritance pattern has been proposed. More rarely, the disease appears to be inherited in an autosomal dominant fashion with reduced penetrance. We are ascertaining and collecting blood from pedigrees showing such an inheritance pattern and combining stringent diagnostic criteria with conservative methods of genetic analysis to positionally clone genes predisposing to IS. Probands having clinically relevant idiopathic scoliosis (50 degree curves or greater) from multiplex families are being identified. Currently DNA samples have been collected from 21 pedigrees containing 154 individuals. A genome-wide scan in one large family (seven affected members of 14 sampled) was conducted with 385 polymorphic microsatellite markers at approximately 10 cM resolution. Affecteds-only linkage analyses, using both parametric and nonparametric methods, produced evidence for linkage with loci on chromosomes 6p, distal 10q, and 18q with maximum non-parametric lod (NPL) scores of 1.42 (p=0.020), 1.60 (p=0.019), and 6.33 (p=0.002) respectively. A genome-wide search was conducted in a second large kindred (seven affected individuals of 17 sampled) using 200 microsatellite markers at an approximate resolution of 25 cM. The same analyses produced maximum evidence for linkage at chromosome 4q and again at distal chromosome 10q, with NPL scores of 5.08 (p=0.015) and 2.55 (p=0.033), respectively. Interestingly, duplication of the common 10q region was recently correlated with the presence of scoliosis (p<.001). The combined data suggest that IS is genetically heterogeneous, but that a limited number of loci may be involved.

Program Nr: 538 from the 1999 ASHG Annual Meeting

Genetic Mapping of a Novel Syndrome of Terminal Osseous Dysplasia and Pigmentary Defects to Human Chromosome Xq27.3-Xqter. *W.Z. Zhang[1,2], R. Amir[2], D.W. Stockton[2], I.B. Van den Veyver[2,3], C. Bacino[2], H.Y. Zoghbi[1,2,4]*. 1) Howard Hughes Medical Institute; 2) Departments of Molecular and Human Genetics; 3) Obstetrics and Gynecology; 4) Pediatrics, Baylor College of Medicine, Houston, TX 77030.

We studied a four-generation family with ten affected females manifesting one or more of the following features: osseous dysplasia of the metacarpals, metatarsals, and phalanges leading to brachydactyly, camptodactyly and other digital deformities; pigmentary defects on face and scalp; and multiple frenulae. There are no affected males and one of the affected females had recurrent spontaneous abortions, consistent with an X-linked dominant male lethal mutation. X inactivation studies using the methylation assay at the androgen receptor locus demonstrated a skewed pattern of X inactivation in seven affected females tested and a random pattern in two unaffected females, thus confirming the X-linked inheritance. To map the gene for this disorder, we undertook genotypic analysis of DNAs from nine affected females and five unaffected individuals (three females and two males) using forty markers evenly distributed throughout the X chromosome. Among the markers tested, thirty-two were informative. Two-point linkage analyses using these markers excluded most of the X chromosome and demonstrated linkage to a region on the long arm from marker DXS548 to Xqter. Assuming 90% penetrance, we obtained a maximum lod score of 3.16 at a recombination frequency of zero for five markers (DXS1123, DXS1113, FRAXF, BGN and DXS1108) in Xq27.3-Xq28. Multipoint linkage analysis confirmed the mapping of this gene to Xq27.3-Xqter. This disorder is similar to the condition reported in a sporadic case by Horii et al (Am J Med Genet. 80: 1-5, 1998) and in cases 2 and 3 by Bloem et al (J Bone Joint Surg [Br]. 56-B (4): 746-51, 1974). In these cases, digital fibromas were a major finding, in contrast to the family we studied where they are prominent in only one female. In summary, we describe and map a novel X-linked syndrome, which should facilitate the identification of the gene causing this disorder.

Program Nr: 539 from the 1999 ASHG Annual Meeting

A genome-wide portrait of short tandem repeats. *C. Zhao, J. Heil, J.L. Weber.* Center for Medical Genetics, Marshfield Medical Research Foundation, Marshfield, WI.

The rapidly growing volume of human genomic sequence offers the opportunity to gain a genome-wide perspective on the relative frequencies, lengths, and organization of short tandem repeats (STRs, also called microsatellites). We have recently completed a computer screen for all mono-, di-, tri-, and tetranucleotide repeats in over 150 mb of human genomic BAC DNA from the Sanger, Washington University, and MIT Sequencing Centers. Emphasis was placed on runs of STRs which are long enough to likely be polymorphic (10 or more uninterrupted repeats for mononucleotides, 12 repeats for dinucleotides, 8 for trinucleotides and 6 for tetranucleotides). Permuted and complementary sequences were combined into alphabetically minimal groups. For mononucleotides (2 types), A repeats were far more common and longer than Cs. For dinucleotides (4 types), AC repeats were strongly predominant over AGs and ATs, and long CG repeats were virtually absent. For trinucleotides (10 types), the most common repeats in order of decreasing frequency were AAT, AAC, AAG, ATC, and AGG. For tetranucleotides (33 types), the top sequences in decreasing frequency were AAAT, AAAC, AAAG, AAGG, AGAT, ATCC, AATG, and AGGG. For longer runs of tetranucleotides with 10 or more repeats, the most frequent in decreasing order were AAAT, AAAG, AGAT, AAGG, and ATCC. When extrapolated to the entire genome, our results indicate that there are likely to be about 1.1 million polymorphic mononucleotide STRs and an additional 200,000 polymorphic di, tri, and tetranucleotide STRs. Since there are thought to be about 3 million total diallelic polymorphisms, our results indicate that STRs comprise a substantial fraction of all DNA polymorphism in the human gene pool. In addition, the approximately 10,000 STRPs developed to date are only a small fraction of available sequences. Enormous numbers of useful STRPs await characterization.

Program Nr: 540 from the 1999 ASHG Annual Meeting

Statistically significant association of sequence variants in the *ABCR* gene with age-related macular degeneration. *R. Allikmets, and the International ABCR Screening Consortium.* Columbia University, New York, NY.

Age-related macular degeneration (AMD) is the leading cause of untreatable central visual impairment among the elderly and has been associated both with environmental and with genetic factors. Previously we have identified disease-associated variants in the *ABCR* gene in a subset of patients affected with this complex disorder. We have now tested our original hypothesis, that *ABCR* is a dominant susceptibility locus for AMD, by screening >1300 unrelated AMD patients of Caucasian origin and >1500 control individuals for the three frequent variants found in *ABCR*. AMD patients and controls, matched by age and/or ethnicity, were independently ascertained in 15 laboratories in the U.S., UK, France, Italy, Spain, Sweden, Germany, and The Netherlands. The *ABCR* gene was screened by either SSCP, DGGE or PCR-RFLP methods for three amino acid-changing variants, G1961E, D2177N, and R943Q. Respective positive controls were included in each experiment and variants were confirmed by direct sequencing. Statistical analysis was performed on accumulated data using Fisher's exact test or c^2 test. G1961E and D2177N mutations were detected in one allele of *ABCR* in 51 patients (~4%), and in 13 controls (~0.9%). Fisher's exact test confirmed that these two variants are associated with AMD at a statistically significant level ($P < 0.000001$). Mutations are found predominantly in patients with the dry (non-neovascular) form of the disorder ($P = 0.0014$). Frequency of the common R943Q polymorphism, used to assess the correlation of patient and control populations, was very similar between patients and controls in all groups (7-10%, $P = 0.23$, with c^2 test). Our data confirms that some *ABCR* variants are associated with AMD phenotype(s). This association is statistically significant when analyzed in large patient/control populations and the two variants alone account for about 4% of all *ABCR* variants associated with AMD. Defining a subset of AMD patients in which heterozygous *ABCR* alterations are associated with the disease will permit presymptomatic testing of high-risk individuals and may lead both to earlier diagnosis of AMD and to new strategies for intervention and prevention.

Program Nr: 541 from the 1999 ASHG Annual Meeting

Single nucleotide variants in XIST: Test of association with X inactivation patterns.. *J.M. Amos-Landgraf, R.M. Plenge, H.F. Willard.* Genetics, Case Western Reserve Univ, Cleveland, OH.

Many X-linked disorders have been reported to manifest to some degree in females. This is likely due to preferential inactivation of the X chromosome carrying the normal allele. The process of X chromosome inactivation (XCI) is believed to be a random event whereby either parental X chromosome has an equal probability of becoming inactivated. The choice of which X chromosome is to be inactivated appears to be random; however, alleles have been identified in inbred mouse strains that control XCI patterns resulting in varying degrees of skewed XCI ratios. These alleles, termed X inactivation controlling element (Xce) alleles, map to the X inactivation center (XIC) and have only been identified in mice. To determine if similar Xce alleles exist in humans, we have identified markers in the X inactivation center (XIC), and are constructing haplotypes to relate these variants to the observed X inactivation ratios. PCR primers were designed utilizing GenBank sequence of PAC 92E23, which contains the XIST gene, and the products directly sequenced . We have sequenced 20 kb of the XIC in 15 unrelated X-chromosomes, identifying 15 single nucleotide changes, and 2 insertion/deletion changes. Nine of the single nucleotide variants (SNVs) were identified exclusively on two unrelated X-chromosomes. Three SNVs have been genotyped in 113 females and have minor allele frequencies of 5% -10%. To determine if these single nucleotide polymorphisms (SNPs) identify potential Xce alleles, genotypes were compared with blood XCI ratios to test for association. There was no significant association of skewed XCI ratios (individuals with ratios of >80:20) with any of the 3 SNPs genotyped, although a previously described variant in the XIST promoter is associated with preferential inactivation (Nature Genetics 1997 vol.17: 353). Extension of this approach to much larger populations of females should uncover possible genetic influences on X inactivation patterns as determined by the XIC and XIST.

Program Nr: 542 from the 1999 ASHG Annual Meeting

Somatic sequence variation at the Friedreich ataxia locus includes complete contraction of the expanded GAA triplet repeat, significant length variation in serially passaged lymphoblasts, and enhanced mutagenesis in the flanking sequence. *S.I. Bidichandani[1], S.M. Purandare[2], E.E. Taylor[1], G. Gumin[1], H. Machkhas[1], Y. Harati[1], R.A. Gibbs[3], T. Ashizawa[1], P.I. Patel[1,3,4].* 1) Neurology, Baylor Col Medicine, Houston, TX; 2) Pathology, Baylor Col Medicine, Houston, TX; 3) Molecular and Human Genetics, Baylor Col Medicine, Houston, TX; 4) Neuroscience, Baylor Col Medicine, Houston, TX.

The majority of Friedreich ataxia patients are homozygous for large GAA triplet repeat expansions in intron 1 of the *X25* gene. Systematic analysis of lymphoblastoid cell lines passaged 20 - 39 times allowed examination of instability of expanded GAA repeats in 23 chromosomes bearing 97 to 1250 triplets. Southern analyses revealed 18 events of significant changes in length ranging from 69 to 633 triplets, wherein the *de novo* allele gradually replaced the original over 1 to 6 passages. Contractions and expansions occurred with equal frequency and magnitude. This behavior is unique in comparison to other large, non-coding triplet repeat expansions ([CGG]n and [CTG]n) which remain relatively stable under similar conditions. We also report a rare patient, who having inherited two expanded alleles, showed evidence of contracted GAA repeats ranging from 9 to 29 triplets in DNA from two independent peripheral blood samples. Furthermore, given that triplexes in transcribed templates cause enhanced mutagenesis, and since the GAA triplet repeat is known to adopt a triplex structure, the poly(A) tract and a 135-bp sequence situated immediately upstream were examined for somatic mutations. The poly(A) tract showed enhanced instability, with a preponderance for deletion when in cis with the GAA expansion. The 135-bp upstream sequence was found to harbor a three-fold excess of point mutations in DNA derived from individuals homozygous for the expansion compared with normal controls. These data are likely to have important mechanistic and clinical implications.

Program Nr: 543 from the 1999 ASHG Annual Meeting

Deletion of the Prader-Willi syndrome imprinting center in mice. *C.I. Brannan, S.M. Blaydes.* Dept Molecular Genetics & Microbiology, University of Florida, Gainesville, FL.

Deletion of a region nearby and including the first exon of the *SNRPN* gene has been shown to cause Prader-Willi syndrome (PWS) upon paternal transmission. This element, dubbed the PWS Imprinting Center (PWS-IC) appears to be responsible for the coordinate expression of multiple paternally expressed genes mapping in the 15q11-q13 region. Previously, we have shown that a similar PWS-IC element exists near the murine *Snrpn* gene and is required for imprint resetting which must occur during gametogenesis. We now demonstrate that the PWS-IC is also required in somatic tissues. Chimeric males derived from male embryonic stem (ES) cell line harboring a deletion of the PWS-IC on the paternal chromosome show abnormal levels of methylation at sites within *Zfp127* and *Ndn*. These results suggest that the PWS-IC is required for the *de novo* establishment and/or maintenance of the paternal imprint. Finally, as progeny derived from germline transmission of this PWS-IC mutation from male chimeras are not viable, it has not been possible to establish a line of mice harboring this deletion. To solve this problem, we have recently deleted the PWS-IC in an XO ES cell line. Female chimeras from this cell line are fertile. Offspring derived from these females will be useful for genetic complementation studies aimed at identifying genes involved in the murine PWS phenotypes.

Program Nr: 544 from the 1999 ASHG Annual Meeting

FMR1 knockout mouse shows a strain-specific severe learning impairment: A robust model for the fragile X syndrome. *W.T. Brown, A. Rabe, R. Dumas, H. Haubenstock, C. Dobkin.* Dept Human Genetics, NYS Inst Basic Research, Staten Island, NY.

Fmr 1 gene knock out (ko) mice are a model for the human Fragile X mental retardation syndrome. However, fmr1 ko mice with a C57BL/6-129/OlaHsd hybrid background have been reported to have only a mild learning deficiency (Bakker et al., 1994). We examined the effect of this ko mutation on spatial learning ability in mice with either an FVB/N-129/OlaHsd hybrid background or a C57BL/6 background. When FVB-129 mice unaffected by the recessive FVB vision impairment mutations were tested, the ko mice showed a profound deficiency in their ability to learn the position of a hidden escape platform in a "cross" water maze in comparison to littermate controls. In contrast, ko mice with a C57BL/6 background learned the maze just as well as their littermates. The C57Bl/6 ko mice did show a mild deficiency in the "reversal" phase of this test, which is a more complex task. FVB/N-129 ko mice also showed differences from control littermates in their hippocampal vs non-hippocampal responses in fear conditioning, differences that were not apparent in C57BL/6 ko mice. Thus, silencing the Fmr1 gene profoundly interfered with specific learning behaviors in FVB/N-129 hybrid mice but only slightly affected mice with a C57BL/6 background. This indicated that the Fmr1 knockout mutation is a robust strain specific model for the human mental retardation syndrome. The clear impairment in FVB-129 ko mice will facilitate testing of potential therapies and investigation of the developmental etiology of the fragile X syndrome. The strain dependence of the ko phenotype may allow the identification of genes that interact with Fmr1.

Program Nr: 545 from the 1999 ASHG Annual Meeting

The *SNRPN* exon 1 region is necessary for the postzygotic maintenance of the paternal imprint. *K. Buiting[1], B. Bielinska[2], C. Lich[1], M. Krajewska-Walasek[2], B. Horsthemke[1].* 1) Institut für Humangenetik, Universitätsklinikum Essen, Essen,Germany; 2) Department of Genetics, The Children's Memorial Health Institute, Warsaw, Poland.

A small subset of patients with Prader-Willi syndrome (PWS) and Angelman syndrome (AS) have apparently normal chromosomes of biparental origin, but the paternal chromosome carries a maternal imprint or the maternal chromosome carries a paternal imprint, respectively. In some of these cases, the incorrect epigenotype is the result of an imprint switch failure in the parental germ line caused by a microdeletion of the imprinting center (IC). In all PWS families studied so far, the IC deletions always included *SNRPN* exon 1 and were on the chromosome inherited from the paternal grandmother.

Recently we identified another PWS patient with a typical IC deletion of about 200 kb. Methylation and microsatellite analyses of blood DNA revealed that the father of the PWS patient is mosaic for the deletion. Since he transmitted the deletion to his affected son, the deletion must also be present in his germ cells, indicating that it must have occurred postzygotically, but prior to the separation of germ cells and somatic cells. In contrast to all other PWS families with IC microdeletions, in this family the affected chromosome is of grandpaternal origin. Unexpectedly, methylation analysis at four loci outside the deletion (*NDN*, u1D, u1B and PW71) showed that the deletion chromosome in both the father and the patient has a maternal methylation imprint. The postzygotic paternal to maternal imprint switch in the father suggests that the *SNRPN* exon 1 region is not only required for the establishment, but also for the maintenance of the paternal imprint.

Supported by the Deutsche Forschungsgemeinschaft.

Program Nr: 546 from the 1999 ASHG Annual Meeting

Identification of Hirschsprung Disease Susceptibility Loci by Linkage Disequilibrium Analysis in the Old Order Mennonites. *M.M. Carrasquillo, A. Chakravarti.* Genetics/Ctr Human Genetics, Case Western Reserve Univ, Cleveland, OH.

Hirschsprung disease (HSCR) is characterized by lack of enteric ganglia and shows a complex pattern of inheritance with mutations in seven different genes (EDNRB, EDN3, ECE1, SOX10, RET, GDNF and NTN), but most mutations show reduced penetrance. A hypomorphic missense mutation, W276C, in the endothelin receptor-B gene (EDNRB) was found in a large number of HSCR cases in the genetically isolated Old Order Mennonite population from Lancaster and Berks Counties, PA. However, W276C is neither necessary nor sufficient for HSCR. To identify additional genes which contribute to Mennonite HSCR we conducted linkage disequilibrium analyses of RET, which showed some association in a genomic screen. We generated and tested 14 new single nucleotide polymorphisms (SNPs) and a novel microsatellite marker in a region of ~ 250 kb encompassing RET. These 15 new markers and 6 previously reported SNPs have an average intermarker distance of ~12 kb. We identified significant association of RET SNPs with respect to chromosomes transmitted and untransmitted to affecteds. Furthermore, haplotype analysis for all markers revealed 5 major haplotypes in this region in 30 families studied. One of the haplotypes, haplotype a, is more often transmitted to affected individuals, suggesting that this haplotype carries a RET susceptibility variant, allowing us to narrow down the genomic region harboring this RET variant to 34 kb conserved identical by descent (IBD) among affected individuals carrying haplotype a. We are sequencing this region to search for the culprit change. In addition, we are conducting additional tests to search for genomic rearrangement in this region of 10q11.2.

Program Nr: 547 from the 1999 ASHG Annual Meeting

Transgenic animals carrying pathological alleles at the MJD1 locus exhibit a mild and slowly progressive cerebellar deficit. *C. Cemal, C. Huxley, A. McGuigan, S. Chamberlain.* Molecular Genetics, Imperial College of Science , Technology and Medicine, London, UK.

The generation of a representative mouse model for Machado-Joseph disease or spinocerebellar ataxia 3 (SCA3) should enhance a greater understanding of the role of repeat length, tissue specificity and level of expression on the pathology of this disease. We have previously reported the generation of human yeast artificial chromosome constructs encompassing the MJD1 locus into which expanded triplet repeat motifs (n=76 or 84) have been introduced by homologous recombination. Transgenic animals subsequently generated using these constructs demonstrate pathological alleles corresponding to a polyglutamine tract length of either 73,74,76 or 84 repeats in the four founders identified to date. The animals characteristically demonstrate a mild and slowly progressive cerebellar deficit, manifesting as early as 4 weeks of age with a slightly kyphotic posture and a fluid but abnormal gait. As the disease progresses, pelvic elevation becomes markedly flattened, accompanied by motor and sensory loss and hypotonia. In contrast, a transgenic animal carrying the wild-type (CAG15) allele at the MJD1 locus appears completely normal at 12 months. The founders show no evidence of early morbidity and exhibit normal growth and fecundity. Although the initial founder (CAG)76 failed to transmit the YAC transgene, transmission to 50% of offspring has been detected in animals carrying alleles corresponding to either CAG73 or CAG84. Further investigation of these animals to elucidate the role of nuclear inclusions in the degenerative process associated with this disorder is currently underway.

Program Nr: 548 from the 1999 ASHG Annual Meeting

Glutathione peroxidase 3: Identification of promoter region polymorphisms and association with age-related maculopathy. *Y.P. Conley[1], M.B. Gorin[1,2], T.S. Mah[2], D.E. Weeks[1], R.E. Ferrell[1]*. 1) Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA 15261; 2) Department of Ophthalmology, University of Pittsburgh, Pittsburgh, PA 15261.

Age-related maculopathy (ARM) is a late-onset, progressive, complex genetic disorder for which oxidative damage has been proposed as a potential etiology. The glutathione peroxidases (GPX) are a family of at least four genes encoding enzymes which catalyze the reduction of hydrogen peroxide, lipid peroxides and organic hyperoxide by glutathione and protect cells from oxidative damage. GPX3 is expressed in a variety of tissues, including eye and brain. To assess the possible role of GPX3 in ARM, we sequenced the five exons and the 5' flanking region of GPX3 from ARM cases and controls (N=10). Two single nucleotide polymorphisms were found in the 5'-flanking region of the gene, an A/G at position -623 and an A/C at position -302. No variation was observed in the coding sequence. The -623G and -302A alleles were in complete linkage disequilibrium. We examined the -623 A/G polymorphism in 518 unrelated ARM-affected individuals (both exudative and nonexudative cases) and 46 individuals age >70 years with no evidence of ARM. The frequency of the A allele was 0.84 among the ARM cases and 0.65 among the controls ($0.001 < p < 0.005$), giving an odds ratio of 2.72 (95% C.I. = 2.1-3.3) for ARM among carriers of the A allele. This result is consistent with the hypothesis that oxidative damage contributes to ARM and that genetic variation in the genes encoding anti-oxidative enzymes may contribute to ARM susceptibility.

Program Nr: 549 from the 1999 ASHG Annual Meeting

A putative imprinting control region in 11p15.5 and its loss of methylation in Beckwith-Wiedemann syndrome.

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Imprinting control elements are proposed to exist within the *KvLQT1*locus since multiple chromosome rearrangements associated with Beckwith-Wiedemann syndrome (BWS) disrupt this gene. We have identified an evolutionarily conserved CpG-island (*KvDMR1*) in an intron of the *KvLQT1*gene which is methylated in the ovary, unmethylated in sperm, and exhibits differential methylation in all human and mouse somatic tissues tested. Strand-specific RT-PCR analysis of the human and syntenic mouse loci demonstrated the presence of a *KvDMR1*-associated RNA transcribed exclusively from the paternal allele and in the opposite orientation with respect to the maternally expressed *KvLQT1* gene. Loss of imprinting at *IGF2*, generally through an *H19*-independent mechanism, is associated with a large percentage of patients with BWS. Among twelve cases of BWS with normal *H19* methylation, five showed demethylation of *KvDMR1* in fibroblast or lymphocyte DNA, while in four cases of BWS with *H19* hypermethylation, methylation at *KvDMR1* was normal. Thus, inactivation of *H19* and hypomethylation at *KvDMR1* represent distinct epigenetic anomalies associated with biallelic expression of *IGF2*. We propose that *KvDMR1* and/or its associated antisense RNA (*KvLQT1-AS*) represents an additional imprinting control element or center in the human 11p15.5 and mouse distal 7 imprinted domains. To test this hypothesis we are carrying out targeted mutagenesis of *KvDMR1* in the mouse and assessing the expression of 11p15.5 imprinted genes in BWS patients with and without loss of methylation at *KvDMR1*. Supported by CA63333.

Program Nr: 550 from the 1999 ASHG Annual Meeting

Expression analysis of ataxin-7 reveals restricted localization of an alternate isoform. *D.D. Einum[1], L. Gouw[1], A. Matilla[2], J. Townsend[3], Y.H. Fu[4], L.J. Ptacek[1,2]*. 1) Dept Human Genetics, Univ Utah, SLC, UT; 2) Howard Hughes Medical Institute, Univ Utah, SLC, UT; 3) Dept Surgical Pathology, Univ Utah, SLC, UT; 4) Dept Neurobiology and Anatomy, Univ Utah, SLC, UT.

Spinocerebellar ataxia type 7 (SCA7) is an inherited disorder characterized clinically by ataxia and vision loss and pathologically by degeneration of cerebellar and brainstem neurons, as well as retinal photoreceptor cells. SCA7 is caused by expansion of a CAG repeat that encodes polyglutamine in the translated protein, ataxin-7. Similar to other polyglutamine disorders, cell death is restricted to specific neurons despite widespread expression of SCA7 transcripts. However, we have identified an alternate SCA7 transcript, termed SCA7.3, that exhibits an expression pattern restricted primarily to the CNS. We hypothesize that cell-specific degeneration in SCA7 is due to polyglutamine expansion within an alternate ataxin-7 isoform exhibiting restricted expression. To examine this possibility, we have developed antibodies and utilized them to examine the expression patterns of distinct ataxin-7 isoforms. Immunoblotting experiments revealed that ataxin-7 is translated, at variable levels, in all peripheral and CNS tissues examined. In contrast, ataxin-7.3 expression is limited to CNS tissues. Furthermore, immunohistochemical staining demonstrated that ataxin-7 and ataxin-7.3 reside in disparate subcellular locales of cerebellar Purkinje cells, neurons which are primarily affected in SCA7 patients. The demonstration that ataxin 7.3 exhibits a tissue distribution restricted primarily to the CNS, as well as a subcellular localization distinct from that of other ataxin-7 isoforms, raises the possibility that the specific pattern of cell death in SCA7 patients is due to polyglutamine expansion within an alternate ataxin-7 isoform. The potential role of ataxin-7.3 in SCA7 pathogenesis is currently under investigation.

Program Nr: 551 from the 1999 ASHG Annual Meeting

Identification of a testis-specific gene in the Prader-Willi syndrome region on chromosome 15. *C. Faerber[1], J. Neesen[2], S. Gross[1], K. Buiting[1], B. Horsthemke[1]*. 1) Institut fuer Humangenetik, Universitaetsklinikum Essen, Essen, Germany; 2) Institut fuer Humangenetik, Universitaet Goettingen, Goettingen, Germany.

Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are caused by the loss of function of imprinted genes within a 2-3 Mbp chromosomal domain in proximal 15q. Whereas Angelman syndrome can be caused by mutations in a single gene (*UBE3A*), which in brain is expressed from the maternal chromosome only, PWS appears to be a contigous gene syndrome. So far, four paternally expressed genes have been identified within the PWS critical region (*ZNF127*, *NDN*, *SNRPN* and *IPW*).

We have identified a novel gene which maps about 300 kb centromeric of *SNRPN*. It was identified by subcloning of a CpG-island close to D15S13 (189-1). The CpG-island was found to be 100% unmethylated in sperm cells, 20% unmethylated in fetal ovary and adult testis, and fully methylated in all other investigated tissues. Northern blot analysis with a probe downstream of the CpG-island revealed a testis-specific transcript of 7.5 kb. Preliminary RNA in situ hybridization data suggest that the gene is expressed in spermatocytes. It is intronless and has an open reading frame of 3.5 kb encoding a 121 kDa protein of unknown function. An unusual polyadenylation signal is provided by a solitary long terminal repeat (LTR) at the 3' end of the gene. Following the HUGO nomenclature, the gene was termed *c15orf2* (chromosome 15 open reading frame 2). To investigate the imprinting status of *c15orf2*, we have identified four polymorphisms within the 3'UTR and analyzed DNA and RNA samples of three adult testes. Interestingly, we found biallelic expression of c15orf2. Therefore, *c15orf2* is the first gene reported to escape from genomic imprinting in the PWS critical region, although the imprinting status of *c15orf2* may be different in earlier developmental stages. It is tempting to speculate that the protein encoded by *c15orf2* may play a role in spermatogenesis.

Part of this work was supported by the Deutsche Forschungsgemeinschaft.

Program Nr: 552 from the 1999 ASHG Annual Meeting

A murine model of triplet repeat instability demonstrates dramatic age dependent, expansion biased somatic mosaicism. *M.Teresa Fortune[1], C. Vassilopolus[1], M.I. Coolbaugh[2], M.J. Siciliano[2], D.G. Monckton[1]*. 1) Division of Molecular Genetics, University of Glasgow, Glasgow, UK; 2) Department of Molecular Genetics, U.T. M.D. Anderson Cancer Center, Houston TX.

Myotonic dystrophy (DM) is one of an increasing number of diseases whose phenotype has been connected to an expansion of a triplet repeat. Expansion over time and variation of the mutation rate between the somatic tissues of an individual is thought to at least partially account for the tissue specificity and progressive nature of the symptoms. The level of instability of the repeat in DM patients is affected by several factors including the initial size of the progenitor allele and the age of the patient. To investigate the behaviour of the expanded CTG repeat in multiple tissues over time, murine models have been developed containing the *Dmt* transgene which is derived from the human DM locus and consists of ~161 CTG repeats. An investigation of instability was carried out on four of the lines generated on a range of somatic tissues using small pool PCR. Initial observation of 20 month old male and female mice from each line revealed minimal levels of instability in three of the lines. In the fourth, *Dmt*-D, expansion biased instability, which varied greatly between tissues, was observed. To further investigate the effect of age on stability on *Dmt*-D, mice were sampled at 2, 6 and 13 months, and instability was found to increase with age as in humans. Stability in regions of the brain from a 12 month old *Dmt*-D mouse was also studied and as with humans and other mouse models only minimal instability was observed in cerebellum. Kidney, the most unstable tissue, demonstrated dramatic instability with expansion events of over 500 repeats observed in old mice. Moreover, the distribution of variability was clearly trimodal, suggesting not just tissue specific, but also cell-type specific modifiers of instability. These mice should provide an excellent model system for further investigating the molecular mechanisms controlling somatic mosaicism at triplet repeat loci.

Program Nr: 553 from the 1999 ASHG Annual Meeting

Multiparameter SNP test identifies polymorphisms in genes from the renin-angiotensin system as predictors of dyslipoproteinemia and, in combination with lipase polymorphisms, of coronary artery disease. *H. Funke[1,2], G. Assmann[1,2], S. Rust[2], H. Schulte[2], E. Köhler[3], M. Grow[4], H. Erlich[4], S. Cheng[4].* 1) Inst Clinical Chemistry, Univ Muenster, Muenster, Germany; 2) Inst Atherosclerosis Res, Univ Muenster, Germany; 3) Salzetalklinik, Bad Salzuflen, Germany; 4) Roche Molecular Systems, Alameda, CA.

In recent years mutations in numerous candidate genes have been proposed as coronary artery disease (CAD) risk modifyers. Often observed conflicting results from different studies are thought to be a consequence of the relatively small contribution to disease formation of a single modifyer gene. Larger effects are expected to result from gene/gene interaction. In an attempt to identify such interaction we have analyzed 34 dimorphic polymorphisms previously suggested as CAD markers in 1000 male myocardial survivors with confirmed CAD and in 1003 male controls from the same region of Germany. Using a c2-test three markers showed genetoype frequencies which differed between the cases and the controls. These markers were apo B 71Ile ([af]=0.329 in cases, [af]=0.300 in controls; $p<0.01$), apo CIII del(T)-625 (af=0.386 vs. af=0.434; $p<0.01$), and ELAM Ser128Arg (af=0.113 vs. af=0.089; $p<0.05$). Subsequent analysis of the association of all 34 SNPs with quantitative intermediate phenotypes (QIP) of CAD identified numerous significant associations. For a more condensed analysis both QIP and SNPs were grouped on the basis of the metabolic pathways they originate from. While gene polymorphisms in genes of the renin angiotensin system (RAS)were associated with changes in blood pressure (ACE ins/del with diast. and syst. BP; $p<0.05$) they also showed a close association with plasma lipoproteins (AGT235 with triglic. and Lp(a); ATIIR1166 with HDL-chol, apoAI, and Lp(a); all $p<0.05$). When RAS polymorphisms and polymorphisms from the lipoprotein lipase (LIPC) gene, neither of which was associated with CAD when tested alone, were analyzed in pairs significant associations with CAD were seen. These data further strengthen the link between high blood pressure and lipoprotein metabolism disorders in the pathophysiology of CAD.

Program Nr: 554 from the 1999 ASHG Annual Meeting

Specific sequence polymorphisms in the *RET* proto-oncogene are over-represented in individuals with Hirschsprung disease and may represent loci modifying phenotypic expression. *O. Gimm[1], S. Borrego[2], M.E. Sáez[2], A. Ruiz[2], M. López-Alonso[3], C. Eng[1], G. Antiñolo[2].* 1) Human Cancer Genetics Program, Ohio State University, Columbus, OH; 2) Unidad de Genética Médica y Diagnóstico Prenatal, Sevilla, Spain; 3) Departamanto de CirugÍa Infantil, Hospital Universitario Virgen del RocÍo, Sevilla, Spain.

Hirschsprung disease (HSCR), a common genetic disorder (1 in 5000 life births), is characterized by functional intestinal obstruction secondary to enteric aganglionosis. Usually occurring as isolated cases, HSCR can be familial with reduced penetrance and male predominance. Even though six putative susceptibility genes have been identified (*RET, GDNF*, *NTN*, *EDNRB*, *EDN3*, *SOX10*), only germline mutations in the *RET* proto-oncogene account for a significant minority (up to 50%) of familial HSCR. In contrast, only 3% of sporadic HSCR in a population-based series carry germline *RET* mutations. We prospectively ascertained 64 incident cases of sporadic HSCR from the Western Andalusia region. We examined allelic frequencies at 7 polymorphic loci within *RET* in this population-based series to determine if polymorphic sequence variants could act as low penetrance predisposing alleles. For two loci, c.135A/G (exon 2) and c.2307C/G (exon 13), the rarer polymorphic allele was over-represented among HSCR cases versus controls. 75 of 128 (59%) HSCR chromosomes tested carried the c.135A/G variant A compared to 32 of 200 (16%) control chromosomes ($p<0.0001$). Further, 39 of 128 (30%) HSCR chromosomes tested carried the c.2307C/G variant C compared to 28 of 200 (16%) control chromosomes ($p=0.0005$). In contrast, 2 other polymorphisms, c.2071G/A (exon 11) and c.2712C/G (exon 15), which are in linkage disequilibrium, were under-represented in the HSCR individuals compared to controls. The polymorphic allele (c.2071A resp. c.2712G) was found in 16 of 128 (12%) HSCR chromosomes compared to 47 of 200 (23%) control chromosomes ($p=0.02$). In conclusion, polymorphisms in the *RET* proto-oncogene appear to predispose to HSCR in a complex, low penetrance fashion and may also modify phenotypic expression.

Program Nr: 555 from the 1999 ASHG Annual Meeting

Genome-wide linkage analyses in the lupus-prone mouse strain, BXSB. *M.E.K. Haywood[1], M.B. Hogarth[1], J.H. Slingsby[1], S.J. Rose[1], E.M. Thompson[2], M.A. Maibaum[1], M.J. Walport[1], B.J. Morley[1]*. 1) Rheumatology Section, Imperial College, Hammersmith, London, U.K; 2) Histopathology, St.Mary's Hospital, London, U.K.

The BXSB mouse strain, derived from an original cross between a C57BL/6 female and an SB/Le male, spontaneously develops a number of phenotypic features which markedly resemble the human disease, systemic lupus erythematosus (SLE). We have established reciprocal backcrosses with the non-autoimmune strain C57BL/10 (B10) and analysed 350 (BXSBx(B10xBXSB)F_1) and 286 (B10x(B10xBXSB)F_1) male backcross mice for a range of phenotypic traits associated with the development of SLE. The mice were genotyped using 93 microsatellite markers and linkage of these markers to disease was studied by extreme phenotype and quantitative trait locus analyses.

We have identified four areas of genetic linkage to disease on chromosome 1 (*Bxs1-4*), a transgressive interval on chromosome 3 (*Bxs5*) and a further interval on chromosome 13 (*Bxs6*) which were associated with various aspects of the phenotype. Additional potential linkages were also identified on chromosomes 2, 8, 10, 11, and 19.

Our analysis suggests that there are multiple genes involved in susceptibility to lupus in BXSB mice. The intervals we have identified segregate with phenotype; *Bxs1* and *Bxs2* were linked to nephritis and splenomegaly, while *Bxs3* was linked to autoantibody production. Disease onset was dependent on BXSB-derived intervals, disease being delayed in backcross mice in comparison to BXSB parental mice. The severity of disease was also linked to BXSB homozygosity, indicating that gene dosage effects are important in this model. There was one exception to this in the backcross to B10, where high levels of antinuclear antibody (ANA) production were linked to B10 derived intervals. The transgressive interval on chromosome 3 provides either a B10 disease susceptibility allele or a BXSB protective allele, and is an excellent candidate locus for modulating ANA levels.

Program Nr: 556 from the 1999 ASHG Annual Meeting

A Mouse Model for Spinal Muscular Atrophy. *H. Hsieh-Li[1], J. Chang[2], Y. Jong[3], H. Li[1].* 1) Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan, ROC; 2) Department of Medical Research, China Medical College Hospital, Taichung, Taiwan,ROC; 3) Department of Pediatrics, Division of Pediatric Neurology, Kaohsiung Medical College, Kaohsiung, Taiwan,ROC.

Homozygous mutation of the telomeric SMN (SMN-T) gene in humans is associated with proximal spinal muscular atrophy (SMA), a severe motor neuron disease leading to muscle weakness with an onset predominantly in infancy and childhood. To further understand the functional role of the SMN gene in SMA, we produced mouse lines carrying a knockout mutation of the mouse Smn gene and transgenic mouse lines that expressed the human centromeric SMN (SMN-C) gene. Mice with the homozygous Smn knockout mutation died during the peri-implantation stage. Transgenic mice harboring the human SMN-C with the Smn gene knockout showed pathological changes in spinal cord and skeletal muscle similar to SMA patients. The severity of the pathology in the knockout-transgenic mice is correlated with the amount of intact SMN protein. The knockout-transgenic mice clearly demonstrate that the SMN-T gene is responsible for SMA and should be useful in elucidating the molecular mechanisms of the SMN gene and in the design of therapeutic protocols for SMA patients.

Program Nr: 557 from the 1999 ASHG Annual Meeting

Quantitative Studies of the Fragile X Mental Retardation 1 (FMR1) Gene Product, FMRP. *A. Kenneson[1], C.H. Hagedorn[2], S.T. Warren[1]*. 1) Departments of Biochemistry, Genetics, and Pediatrics, Emory University, Atlanta, GA, and Howard Hughes Medical Institute, Atlanta, GA; 2) Department of Medicine, Emory University, Atlanta, GA.

The Fragile X Mental Retardation 1 (FMR1) protein product, FMRP, is an RNA-binding protein which is necessary for normal cognitive function, as the lack of functional FMRP results in fragile X syndrome. Within cells, FMRP shuttles between the nucleus and the cytoplasm, where it is associated with translating polyribosomes. Using purified flag-tagged murine FMRP expressed in Sf9 cells as a standard, we developed the first quantitative assay for the accurate measurement of FMRP levels. The assay is slot blot-based, and uses the anti-FMRP antibody, MAb1a. Two experiments using this assay are described here. In the first, FMRP levels in EBV-transformed human B cells were measured relative to eIF4e, the rate-limiting factor in eukaryotic translation. eIF4e levels were quantified using purified human eIF4e as a standard. The molar ratio of eIF4e:FMRP was 3.38 (se=0.09, N=8), with 2.87×10^6 (se=0.13×10^6, N=8) molecules of FMRP per cell, and 9.68×10^6 (se=0.39×10^6, N=8) molecules of eIF4e per cell. The low level of FMRP relative to other components of the translation machinery is consistent with the hypothesis that FMRP is involved in the regulation of a subset of mRNAs, rather than in global regulation of translation.

The assay was also used to quantify FMRP levels in lymphocytes isolated from peripheral blood samples. The amount of FMRP in these specimens does not vary greatly between individuals: 2.24×10^{-15} moles of FMRP per microgram of total cellular protein (se=0.13×10^{-15}, N=9). This suggests that FMRP levels are tightly regulated in lymphocytes, and raises the possibility that FMRP quantification may be performed on peripheral blood samples. The quantitative nature of this assay makes it ideal for the accurate measurement of FMRP in patients with potential reductions of FMRP levels, and in female carriers of full mutations.

Program Nr: 558 from the 1999 ASHG Annual Meeting

The peroxisome-proliferator-actived receptorg2 gene codon 12 mutation in early-onset extremely obese individuals: a gain of function mutation? *W.D. Li, J.H. Lee, R.A. Price.* Behavioral Genetics Laboratory, Department of Psychiatry, Univ Pennsylvania Medical Center, Philadelphia, PA 19104.

The Peroxisome Proliferator Activated Receptor g2 (PPARg2), a nuclear transcription regulator, is a key factor in adipocyte differentiation. Several mutations of human PPARg2 gene have been found in obese patients, some associated with lower and others with higher body mass index (BMI). **Methods:** To study the role of PPARg2 codon 12 mutations (Pro/Ala) in human obesity, we screened for sequence variants in 243 unrelated obese (BMI>27kg/m^2, including 208 extremely obese with BMI>40kg/m^2) individuals, and 157 normal weight controls (BMI<27kg/m^2) using PCR-SSCP and sequencing. We compared the mutation frequencies in obese and normal weight groups and detected associations between codon12 mutation and several obesity related phenotypes including obesity status, age of onset, fasting glucose and NIDDM. **Results:** 1) In the obese group (N=243), the frequency of codon 12 mutations (63/243, 25.93%) was higher than in normal weight (21/157, 13.38%) individuals (c^2=9.06, p=0.0026). The results remained significant when restricted to Caucasian women (c^2=5.03, p=0.0249). In all 400 obese and normal weight individuals, adjusted by age and sex, logistic regression showed an association of codon 12 and obesity affection status (odds ratio 2.3, 95% CI 1.3-3.9). 2) Among 243 unrelated obese individuals, the number of copies of the codon12 mutation predicted age of onset (Pro/Pro, 14.12±10.00; Pro/Ala, 10.15±8.41; Ala/Ala, 7.75±8.26, t=-2.719, p=0.0072). 3) The codon 12 mutation also was associated with lower fasting glucose (Pro/Pro 6.27±3.51mmol/L, Pro/Ala+Ala/Ala 4.93±1.19mmol/L, P<0.001) in the obese group. 4) A marginally significant association was found between the Pro/Pro genotype and NIDDM. **Conclusion:** The association of the PPARg2 codon12 mutation with a higher level of obesity, earlier age of onset, lower serum glucose and NIDDM are consistent with the known effects of PPAR g on adipocyte differentiation and the mediation of PPARg of thiazolidinedione (TZD) in the treatment of diabetes. Functional analysis of PPARg2 is in progress and will be reported.

Program Nr: 559 from the 1999 ASHG Annual Meeting

Genome scan identifies a locus affecting gamma globulin expression in human beta-cluster YAC transgenic mice.
S.D. Lin, P. Cooper, E.M. Rubin. Genome Science Department, Lawrence Berkeley National Lab, Berkeley, CA.

Disorders of beta-globin structure and expression represent among the most common Mendelian inherited causes of disease. Genetic factors affecting gamma-globin gene expression, a major modifier of the severity of both beta-thalassemia and sickle cell anemia, have been difficult to study in mice since the mouse has no equivalent to human gamma globin. To genetically screen for loci affecting gamma expression, we have created YAC transgenic mice containing the entire human beta-globin gene cluster, including the gamma globin gene. Since it has been shown that human gamma-globin transgenes are inactivated prenatally in mice, the 240 kb YAC transgene used for these studies contained a Greek hereditary persistence of fetal hemoglobin (HPFH) allele. The Greek HPFH allele differs from wild type by a single base G to A in the gamma promoter and results in the production of 5-10% gamma globin levels in both humans and transgenic mice into adulthood. FVB mice containing the human transgene were crossed with several different inbred strains and human gamma/beta globin ratio assessed at 10, 15, 30 and 60 days after birth in the various F1 hybrid transgenic mice. The gamma/beta globin ratio of the C3HeB/FVB hybrid transgenics was significantly elevated compared to the FVB transgenics throughout the sampling period. To map the loci involved in regulating gamma globin expression we generated 183 backcross transgenic animals. A 20 cM genome scan followed up by more densely spaced marker analysis of promising loci was performed to map loci affecting gamma globin expression. The most promising locus was mapped to a 4.4 cM interval of chromosome 1 with lod score of 4.3. Work is underway to fine map and clone this gene. Combining the transgenic modeling of a long studied disease-causing human gene cluster with quantitative trait analysis has resulted in the identification and mapping of a locus that impacts on gamma globulin expression.

Program Nr: 560 from the 1999 ASHG Annual Meeting

Search for autism susceptibility loci: genome screen follow-up and fine mapping of a candidate region on chromosome 7q. *E. Maestrini[1], International Molecular Genetic Study of Autism Consortium[2]*. 1) Wellcome Trust Centre, Univ Oxford, Oxford, England; 2) http://www.well.ox.ac.uk/~maestrin/iat.html.

Autism is a neurodevelopmental disorder with a strong genetic component, characterised by impairments in reciprocal social interaction and communication, and restricted and stereotyped patterns of interests and activities. The genetics of the disorder is complex, probably involving the action of several genes. A two-stage genome screen for autism susceptibility loci has been recently completed by the International Molecular Genetic Study of Autism Consortium (IMGSAC, 1998). In the first stage 39 families were typed with 354 markers, and in the second stage 60 additional families were typed using a subset of 175 markers that focused on the interesting regions identified in stage 1. We identified regions on 6 chromosomes (4,7,10,16,19 & 22) each with a maximum multipoint lod score (MLS) greater than 1. A region on chromosome 7q was the most significant (MLS=3.5 in the UK families subset). The second stage of the genome screen has now been completed by typing all 99 families with the collection of 354 markers. The final results confirm the previously reported findings, with the chromosome 7 locus remaining the most significant; in addition regions on chromosomes 1,2,9 & 17 have been identified with an MLS greater than 1. All regions providing an MLS greater than 1 are currently being investigated in a further set of 60 relative-pair families. Other independent studies also reported suggestive evidence of linkage in the 7q region, suggesting that this locus may have a considerable genetic effect in autism. We have further analysed the 7q region by genotyping 51 additional markers in 131 families, including 14 novel microsatellites from candidate genes. The new data provided further support for linkage with an MLS of 4.45 in all families. Linkage disequilibrium mapping is currently being carried out with microsatellite and single nucleotide polymorphism markers using the transmission disequilibrium test. Preliminary evidence of association will be presented. Candidate genes which map close to the linkage peak are also currently being screened for mutations in autistic patients.

Program Nr: 561 from the 1999 ASHG Annual Meeting

Co-localization of CBP with expanded polyglutamine-containing androgen receptor. *A.R.V. McCampbell[1], A.A. Taye[1], J.L. Walcott[3], D.E. Merry[2], K.H. Fischbeck[1].* 1) Neurogenetics Branch, NINDS, Bethesda, MD; 2) Department of Neurology, University of Pennsylvania, Philadelphia, PS; 3) Department of Pharmacology, University of Pennsylvania, Philadelphia, PA.

Spinal and bulbar muscular atrophy is a neurodegenerative disorder caused by a polyglutamine expansion in the androgen receptor (AR). At least seven other disorders, including several spinocerebellar ataxias and Huntingtons disease, share this type of mutation. A pathological hallmark of these diseases is the formation of intranuclear inclusions. These inclusions are thought to be seeded by the polyglutamine-containing portion of the mutated gene product. While the specific role of the inclusions remains controversial, nuclear localization of the mutant protein has been implicated in the neuronal toxicity of expanded polyglutamines. This has prompted our search for nuclear factors whose disruption could be detrimental to the cell. Recent work has demonstrated that the CREBbinding protein (CBP) interacts with normal androgen receptor. CBP, an important mediator in multiple signaling pathways, may be present in limiting quantities. Given that CBP interacts with the N-terminal region of the androgen receptor and its importance in intracellular signaling, we have looked at the distribution of CBP in cells expressing truncated forms of the androgen receptor with varying lengths of polyglutamine repeats. In a mouse motoneuronneuroblastoma fusion cell line, those cells with intranuclear inclusions showed a redistribution of CBP to the inclusion. Similar results were obtained in transgenic mice expressing the same AR constructs driven by the PRP promoter. These data suggest that CBP sequestration by expanded polyglutamine-containing AR may be a mechanism of neuronal toxicity in this disease.

Program Nr: 562 from the 1999 ASHG Annual Meeting

Localization of DNA replication origins at human triplet repeat loci by Kinetic PCR. *T. Nenguke, N. Arnheim.* Molecular Biology, 'Univ Southern California, Los Angeles, CA.

Instability of trinucleotide repeats is believed to be due to their ability to form unusual DNA structures, possibly during DNA replication. Triplet repeats cloned into bacterial plasmids have been shown to stall replication forks most likely by formation of secondary structures in a manner dependent on the orientation of the repeat tract with respect to an origin of DNA replication. Studies in yeast have shown that instability of trinucleotide repeat tracts is also orientation dependent. We have set out to correlate the positions and proximities of origins of DNA replication with respect to the wide range of instabilities observed in the different human trinucleotide repeat diseases. DNA from unsynchronized human tissue culture cells was isolated and short nascent strands (0.5-1.5 kb) were obtained by extrusion, followed by neutral-sucrose gradient centrifugation and agarose gel electrophoresis. This nascent DNA was subsequently used as template for Kinetic PCR using primer pairs set at uniform intervals throughout the candidate region. Kinetic PCR can quantitate the number of starting template molecules for each target region. The primer pairs giving the largest amount of PCR product must be close to an origin of DNA replication. We were able to quantitate the accumulation of PCR product for primer pairs covering non-repetitive sequences in a 40 kb human genomic sequence which includes the HD gene. To date we have localized an origin of replication at the HD gene locus close to the triplet repeats. Studies to localize the position of replication origins and the orientation of the triplet repeats at several other disease loci are currently underway.

Program Nr: 563 from the 1999 ASHG Annual Meeting

Effects of FBN2 mutations on fibrillin-1 deposition in the extracellular matrix of CCA fibroblast cell strains. *E.A. Putnam[1], I. Kaitila[2], D.M. Milewicz[1]*. 1) Dept Internal Med/Div Med Gen, Univ Texas Medical Sch, Houston, TX; 2) Helsinki Univ Central Hosp, Finland.

Congenital contractural arachnodactyly (CCA) is an autosomal dominant disorder similar to Marfan syndrome (MFS). CCA results from mutations in FBN2 encoding fibrillin-2 (fib-2), while MFS results from mutations in FBN1 encoding fibrillin-1 (fib-1). Both proteins are found in 10-12 nm microfibrils in the extracellular matrix (ECM), but it is unknown if fib-2 and fib-1 are found in heterogeneous microfibrils, or if the proteins segregate to individual microfibrils. Dermal fibroblast cell strains were used to examine the effect of a FBN2 mutation on fib-containing microfibril deposition, even though this cell type produces primarily fib-1. A fibroblast cell strain was analyzed, containing an unusual deletion/insertion mutation in the genomic DNA of a patient with classic familial CCA. This mutation resulted in the replacement of exon 24 by exon 23 in the mRNA, predicting the replacement of the 8-cysteine domain by an EGF-like domain. Pulse-chase analysis of the fib produced by the patient fibroblast strain demonstrated that normal amounts of fib were synthesized, secreted, and processed by these cells, but that there was a decrease in fib deposition into the ECM. Similar results were obtained from the analysis of cells established from the patient's affected father. Immunoprecipitation analysis using antibodies specific for fib-1 and fib-2 showed approximately 40 times more fib-1 produced than fib-2. Using fib-1 specific antibodies, immunofluorescence analysis of the mutant cell strains confirmed that the amount of fib-1 containing microfibrils was reduced compared to control cells, similar to results from MFS cell strains. Pulse-chase analysis of 7 other CCA fibroblast strains with different characterized FBN2 mutations showed decreased fib-1 ECM deposition in 5 of these strains. These results demonstrate that FBN2 mutations have an effect on fib-1 deposition into the ECM, indicating that fib-1 and fib-2 interact to form microfibrils. The similarities in CCA and MFS phenotypes may result from disruption of fib-1 microfibrils by mutant fib-2.

Program Nr: 564 from the 1999 ASHG Annual Meeting

Clinical and pathologic characterization of epilepsy in patients with monosomy 1p36, and the search for candidate genes. *S.K. Shapira, H.A. Heilstedt, D.E. Starkey, D.L. Burgess, A.K. Chedrawi, A.E. Anderson, B. Tharp, C.L. Carrol, D. Armstrong, Y.-Q. Wu, J.L. Noebels, L.G. Shaffer.* Baylor College of Medicine, Houston, TX.

Monosomy 1p36 is a recently recognized chromosomal deletion syndrome with a spectrum of clinical features, including hypotonia, psychomotor retardation, and seizures. These neurologic features of the syndrome often lead to the initial cytogenetic evaluation. In the current study, an extensive clinical evaluation was performed on 21 patients with monosomy 1p36, in which most had a neurologic exam, EEG, brain MRI scan, and molecular delineation of the deletion size. All patients had hypotonia and developmental delay, and 95% (20/21) had moderate-to-severe mental retardation. Seizures occurred in 48% of patients (10/21) and epileptiform abnormalities on EEGs were present in 37% (7/19). Brain MRI scans showed leukoencephalopathy in 76% of patients (13/17). Postmortem neuropathologic evaluation in one case demonstrated extensive neuronal and glial heterotopias, and polymicrogyria. To evaluate for the molecular basis of epilepsy in these patients, the candidate gene approach was used. Recent studies indicate that mutations in some potassium (K^+) channel genes result in susceptibility to epilepsy or other neurologic abnormalities. The K^+ channel b subunit gene, *KCNA2B,* which has sequence homology to the *Shaker* subfamily of *Drosophila* voltage-gated K^+ channel genes, had been previously localized to 1p36. PCR primers based on the $3^{'}$ untranslated region of human *KCNA2B* were used to screen a BAC library, yielding one positive clone. Evaluation of this BAC by STS content mapping showed that it also contained marker D1S2633, thus placing *KCNA2B* on the physical map. FISH analysis of lymphoblastoid cell lines with the BAC DNA, demonstrated that *KCNA2B* was deleted in 5 of the 19 patients studied by EEG; all 5 had epileptiform EEGs and seizures. Two patients with epileptiform EEGs who were not deleted for the BAC are under further investigation for candidate loci. These results suggest a strong correlation between deletion of *KCNA2B* and epileptiform abnormalities on EEGs in patients with monosomy 1p36.

Program Nr: 565 from the 1999 ASHG Annual Meeting

A potential autism susceptibility marker on chromosome 15 includes a genomic deletion at D15S822. *J.S. Sutcliffe[1], E.L. Nurmi[1], J.L. Haines[1], J. Piven[2], S.E. Folstein[3], and the Collaborative Linkage Study of Autism[1,2,3]*. 1) Program in Human Genetics, Vanderbilt Univ Medical Center, Nashville, TN; 2) UNC Developmental Disabilities Research Center, Charlotte, NC; 3) New England Medical Center, Boston, MA.

Autism is a neurodevelopmental disorder characterized by deficits in social interaction and language development and repetitive or stereotyped behaviors. Neurological findings may include mental retardation, epilepsy, hypotonia, motor sterotypies such as hand flapping, and self-injurious behaviors. While autism has a complex etiology, evidence from twin and family studies indicates a strong genetic component. Chromosome 15q11-q13 is implicated in autism based on observations of chromosomal duplications and evidence for linkage and increased recombination in the autistic population. Screening for autism susceptibility loci in multiplex families by the Collaborative Linkage Study of Autism (CLSA) project identified several instances of informative null alleles at marker D15S822 in a region where linkage and excess recombination have been detected. Southern analysis at the marker locus revealed common variant fragments, consistent with deletion junctions, in all individuals with null alleles. PCR amplification and sequence analysis of a deletion-specific fragment indicates the deletion is ~5-kb and identical in unrelated individuals. To determine whether the deletion has any statistical relevance to autism, a duplex PCR assay was developed to amplify deleted and nondeleted chromosomes, and this was applied to the CLSA multiplex and simplex autism patient set and controls. The null allele frequency in unrelated individuals was 10/545 (1.8%) chromosomes in the autism population and 2/606 (0.3%) in unrelated control chromosomes, with $p = 0.017$, suggesting it is a potential susceptibility marker in the autism population. It is unclear whether this deletion has a functional effect on any gene, although BLAST analysis identified multiple ESTs representing three exons encoding an apparently novel gene located 8 kb from the deletion. Northern analysis reveals expression of a moderate-abundance, testis-specific transcript of 1 kb.

Program Nr: 566 from the 1999 ASHG Annual Meeting

Early onset Non Insulin Dependent Diabetes Mellitus (NIDDM) - a link with paternal duplication of 6q24 and Transient Neonatal Diabetes Mellitus (TNDM). *I.K. Temple[1], R.J. Gardner[2], D.J.G. Mackay[2], D.O. Robinson[2], G. Valerio[3], A. Franzese[4], J.C.K. Barber[2], J.P.H. Shield[5]*. 1) Wessex Clinical Genetics Service, Princess Anne Hospital, Southampton, Hampshire, UK; 2) Wessex Regional Genetics Laboratory, Salisbury Health Care NHS Trust, Salisbury, UK; 3) Department of Pediatrics, University of Udine Medical School, Udine, Italy; 4) Department of Pediatrics, University Frederico 11, Naples, Italy; 5) Department of Child Health, Institute of Child Health, Bristol, UK.

Transient Neonatal Diabetes Mellitus (TNDM) is a rare disorder with an incidence of 1 in 400,000. The gene has been mapped to chromosome 6q24 and is predicted to be imprinted. We have performed a clinical and molecular study of 30 patients with TNDM and their relatives. We were able to divide the patients into three aetiological groups and no significant clinical differences were demonstrated between them:- Group 1 : Paternal uniparental isodisomy of chromosome 1 was found in 11 cases (37%). There was no significant family history. Group 2: A paternal duplication of chromosome 6q24 was present in a further 11 patients (37%)and accounted for all familial cases. Two relatives from two pedigrees were found to have developed type 2 diabetes; a paternal cousin during her first pregnancy and a sibling in his teenage years. Neither individual had an early history of TNDM. Both were shown to carry a paternally inherited 6q duplication. It was demonstrated using fluorescent PCR, was not visible using standard cytogenetic techniques and was apparently identical to that found in the proband. Group 3: In 8 TNDM patients no molecular anomaly has yet been demonstrated. NIDDM was reported in 4 maternal grandparents. This represents the largest cohort of TNDM patients to be studied and the molecular cause was determined in 74% of cases. The findings of early onset NIDDM in relatives of TNDM patients broadens the clinical spectrum of the disorder. It also lends support to the possibility that the gene for TNDM may have implications for more common types of diabetes.

Program Nr: 567 from the 1999 ASHG Annual Meeting

Mutations in a novel gene encoding a RBCC protein underlie Mulibrey Nanism. *K. Avela[1], N. Idänheimo[1], M. Lipsanen-Nyman[2], E. Seemanová[3], S. Rosengren[4], J. Perheentupa[2], A. de la Chapelle[5], A.-E. Lehesjoki[1]*. 1) Folkhälsan Institute of Genetics and Department of Medical Genetics, University of Helsinki, 00280 Helsinki, Finland; 2) Childrens Hospital, University of Helsinki, 00290 Helsinki, Finland; 3) Institute of Biology and Medical Genetics, 2nd Medical School, Charles University, 150 06 Prague-Motol, Czech Republic; 4) Childrens Hospital, Connecticut, USA; 5) Division of Human Cancer Genetics, Department of Medical Microbiology and Immunology, Comprehensive Cancer Center, Ohio State University, Columbus, Ohio 43210, USA.

Mulibrey Nanism (MUL; MUscle-LIver-BRain-EYe Nanism; MIM 253250) is an autosomal recessive disorder in which the clinical presentation is pleiotropic involving multiple tissues of mesodermal origin. Typical clinical features include growth failure of prenatal onset, dysmorphic features, constrictive pericardium with consequent hepatomegaly, yellowish dots in the ocular fundi, and a J-shaped sella turcica. The incidence of MUL is highest in Finland, and the reported non-Finnish MUL patients are mostly sporadic cases. We have previously assigned the MUL gene by linkage and linkage disequilibirium analysis to chromosome 17q and constructed a physical contig of bacterial clones over the critical MUL region. Here we report the identification of the MUL gene using a positional candidate gene approach. We have identified four different mutations in a gene that encodes a novel RBCC protein. Our data indicate that mutations in this novel gene cause the multi-organ phenotype, MUL.

Program Nr: 568 from the 1999 ASHG Annual Meeting

Multiple *CYP1B1* mutations and incomplete penetrance in an inbred population segregating Primary Congenital Glaucoma suggest frequent *de novo* events and a dominant modifier locus. *B.A. Bejjani[1], D.W. Stockton[1], R.A. Lewis[1,2], K.F. Tomey[2], D.K. Dueker[2], M. Jabak[2], W.F. Astle[2], J.R. Lupski[1]*. 1) Baylor College of Medicine, Houston, TX; 2) King Khaled Eye Specialist Hospital, Saudi Arabia.

Primary Congenital Glaucoma (PCG) is an autosomal recessive disorder caused by unknown developmental defect(s) of the trabecular meshwork and anterior chamber angle. Recently, we reported three distinct mutations in *CYP1B1*, the gene for cytochrome P4501B1, in 25 Saudi families segregating PCG. In this report 37 additional families were analyzed and the initial finding of decreased penetrance confirmed. Mutations and intragenic single nucleotide polymorphisms (SNPs) were also analyzed after direct sequencing of *CYP1B1* coding exons. Eight distinct mutations were identified: G61E, R469W, and D374N, the most common Saudi mutations, account for 72%, 12% and 7% of the PCG chromosomes, respectively. Five additional homozygous mutations (two deletions and three missense mutations) were detected, each in a single family. Affected individuals from 5 families had no *CYP1B1* coding region mutations, and each had a unique SNP profile. The identification of eight distinct mutations in a single gene, against at least four distinct haplotypes, suggests a relatively recent occurrence of multiple mutations in *CYP1B1* in Saudi Arabia. The data presented demonstrate decreased penetrance for the PCG phenotype in the Saudi population, because 38 apparently unaffected individuals from 20 families, two of whom were subsequently diagnosed with glaucoma, have mutations and haplotypes identical to their affected siblings. Two other individuals had abnormal ocular findings that are consistent with glaucoma. Analysis of these 20 kindreds suggests the presence of a dominant modifier locus that is not genetically linked to *CYP1B1*. Linkage and Southern analyses excluded a number of functional candidate modifier loci.

Program Nr: 569 from the 1999 ASHG Annual Meeting

Functional characterization of the Opitz syndrome gene product (midin): evidence for homodimerization and association with microtubules throughout the cell cycle. *S. Cainarca, S. Messali, A. Ballabio, G. Meroni.* Telethon Inst Genetics & Med, Milan, Italy.

Opitz syndrome (OS) is a multiple congenital anomaly manifested by abnormal closure of midline structures. The gene responsible for the X-linked form of this disease, MID1, encodes a protein (midin) that contains a RING, two B-boxes, a coiled-coil (the so-called tripartite motif) and an RFP-like domain. The tripartite motif is characteristic of a family of proteins, named the B-box family, involved in cell proliferation and development. Since the subcellular compartmentalization and the ability to form multiprotein structures both appear to be crucial for the function of this family of proteins, we have studied these properties on the wild-type and mutated forms of midin. We found that endogenous midin is associated with microtubules throughout the cell cycle, colocalizing with cytoplasmic fibers in interphase and with the mitotic spindle and midbodies during mitosis and cytokinesis. Consistent with the evidence obtained by gel filtration analysis, immunoprecipitation experiments demonstrated the ability of the tripartite motif to mediate midin homodimerization, showing that midin exists in the form of large protein complexes. Single domain deletions revealed the coiled-coil as essential for homo-interactions while the other domains are probably necessary to mediate interaction with proteins other than midin. Preliminary data suggest that hetero-interaction can occur with members of the B-box family and with other proteins identified through a two-hybrid screening. Functional characterization of altered forms of midin, resulting from mutations found in OS patients, revealed that association with microtubules is compromised, while the ability to homodimerize and form multiprotein complexes is retained. We suggest that midin is involved in the formation of multiprotein structures acting as anchor points to microtubules and that impaired association with these cytoskeletal structures causes Opitz syndrome developmental defects.

Program Nr: 570 from the 1999 ASHG Annual Meeting

Identification and characterization of 4 large (1.4 kb-39 kb) deletions in TSC2 using long range PCR suggests diverse deletion mechanisms. *S.L. Dabora[1,2,3], D. Franz[4], S. Jozwiak[5], D.J. Kwiatkowski[1,2,3]*. 1) Div. of Hematology, Brigham & Women's Hospital, Boston, MA; 2) Dept. of Adult Oncology, Dana-Farber Cancer Institute, Boston, MA; 3) Harvard Medical School, Boston, MA; 4) Div. of Pediatric Neurology, Children's Hospital medical Center, Cincinati, OH; 5) Dept. of Child Neurology, Children's Memorial Hospital, Warsaw, Poland.

Large deletions ranging in size from several hundred base pairs to over 100 kb account for an estimated 20% of all TSC2 mutations (Jones et al., 1999; Am. J. Hu. Gen. 64:1305-1315). We have developed a long range PCR-based method for detecting large deletions in TSC2 to avoid the limitations and difficulties associated with Southern blotting, pulsed-field gel electrophoresis and fluorescence in-situ hybridization analysis . A series of PCR primers were designed such that 13 overlapping genomic segments of the TSC2 gene are amplified, ranging in size from 2.6 kb to 12.7 kb. Agarose gel electrophoresis is then used to detect deletions. Additional PCR reactions are performed using subsets of these primers such that a single forward primer 5 to the first exon is combined with a collection of 6 reverse primers spanning the TSC2 genomic region and located between 15 kb and 40 kb from the forward primer. In this nested, multiplex reaction, amplification occurs only in the presence of a large deletion in TSC2. This series of 14 PCR reactions have been used to screen 50 TSC patient samples (partially screened for small mutations in TSC1 and TSC2) and large deletions have been detected in 4 of these. The deletions range in size from 1.4 kb to 39 kb and the deletion junctions of each have been sequenced. In one case, Alu-mediated recombination appears to be the mechanism of deletion. In the second case, there is a 3 base pair sequence shared at the deletion junction which may have led to illegitimate recombination. In the last two cases, there is no homology at the deletion junctions. In one of these a 10 bp duplication of the end sequence occurred. In the other, 6 random bases were inserted at the deletion junction. We are extending this analysis using additional primer combinations.

Program Nr: 571 from the 1999 ASHG Annual Meeting

Genetic evidence for cooperative interaction of polycystins 1 and 2, which are mutated in polycystic kidney disease (ADPKD). *C.C. Deltas[1], R. Mean[1], G. Kyriakides[2], M. Hadjigavriel[2], K. Demetriou[3], A. Pierides[3], M. Koptides[1].* 1) Dept Molecular Genetics, Cyprus Inst Neurology & Gen, Nicosia, Cyprus; 2) Paraskevaidion Surgical and Transplantation Center; 3) Dept of Nephrology, Nicosia General Hospital, Nicosia, Cyprus.

Mutations in the PKD1 and PKD2 genes are responsible for adult onset ADPKD1 or 2. The gene products, polycystins 1 and 2, are membranous glycoproteins with extracellular and short cytoplasmic domains. The large extracellular domain of polycystin 1 is predicted to act as a receptor to an unknown ligand which transmits signals from the extracellular environment to the cell. A downstream effect may be the opening of a Ca++ ion channel, polycystin 2. Previously, we showed that focal cyst formation in ADPKD2 may be the result of somatic mutations which inactivate the wild type allele of polycystin 2 (Koptides et. al, 1999, Hum Mol Genet 8: 509-513). Here, we investigated the hypothesis that renal epithelial cells of a patient with ADPKD1, become cystic because of the occurrence of somatic mutations in either the PKD1 or the PKD2 gene. By linkage analysis we showed that ADPKD in family CY1609 was due to a germinal mutation in the PKD1 locus. During a transplant operation of a patient, one kidney was resected and used for further experimentation. We isolated DNA from epithelial cells lining the inner wall of 23 large well separated cysts. We tested the PKD1 and the PKD2 genes for loss of heterozygosity (LOH) using flanking and intragenic markers, and for mutations using the SSCP screening method. LOH was detected in a single cyst for the wild type PKD1 gene with marker SM6. Screening of the PKD2 gene revealed that three cysts had the same mutation we had reported previously (197-203insC). Also, three other cysts showed unique SSCP variants that are presently under investigation. Our data support the hypothesis that somatic mutations in either PKD gene can trigger cyst formation on the background of an inherited PKD1 defect. Also, we suggest that regulation of normal kidney development requires that polycystins 1 and 2 are present at a minimum threshold level. Funded by the Cyprus Kidney Association and CYPRUS TELETHON 98.

Program Nr: 572 from the 1999 ASHG Annual Meeting

Gene targeting of dystrophin Dp260 isoform. *R. Gaedigk[1], K.M. Fitzgerald[1], S.A. Giambrone[1], G.W. Cibis[1], D.M. Pinson[2], W.J. Brunken[3], R.A. White[1].* 1) Genetics, The Children's Mercy Hospital, Kansas City, MO; 2) Department of Pathology, Univ. of Kansas School of Medicine, Kansas City, KS; 3) Massachusetts General Hospital and Harvard Medical School, Charlestown, MA.

Dystrophin is the largest identified human gene and spans at least 2.4 Mb on chromosome Xp21. It is known to be differentially expressed. Several isoforms have been identified with at least seven different promoters regulating transcription. The Dp260 isoform is preferentially expressed in retina and to lower amounts in most other tissues. Dp260 includes a specific first exon located at the 3' end of intron 29. An abnormal electroretinogram (ERG) observed in boys with Duchenne muscular dystrophy (DMD) is thought to be caused by a lack of Dp260. To learn more about the function of this isoform we performed a knockout experiment by replacing this specific R1 exon with the neomycin gene without interrupting the reading frame of the full length Dp427 as well as the remaining dystrophin isoforms Dp140, Dp116 and Dp71. A pathological analysis of the generated Dp260 deficient mice showed no identifiable phenotypic abnormality in kidney, liver, spleen, testis, brain, lung, muscle, heart, GI tract and bone. However, electroretinogram recordings on Dp260 knock-out mice resulted in increased b-wave implicit time in Dp260-null mice. ERG analyses were also performed for normal, *mdx*, and *mdx*^{Cv3} mice. Neither the Dp260-null or *mdx*^{Cv3}mice demonstrate the negative ERG phenotype seen in boys with DMD. This could represent a difference in retinal circuitry between species. The dystrophin isoform Dp260 is responsible in part for the abnormal ERGs seen in DMD. Immunohistochemistry of the retina of Dp260 deficient mice will provide further insight into the function of this isoform. ^{Cv3}.

Program Nr: 573 from the 1999 ASHG Annual Meeting

Desmin gene mutations in myofibrillar myopathy. *L.G. Goldfarb, K.-Y. Park, C. Semino-Mora, H.-S. Lee, S. Litvak, K. Sivakumar, M.C. Dalakas.* Clinical Neurogenetics Unit and Neuromuscular diseases section, MNB/NINDS/NIH, Bethesda, MD 20892.

Myofibrillar myopathy has been defined as amorphous accumulation of myofibrillar proteins in the sarcoplasm of muscle fibers. The disease is expressed as proximal and distal limb muscle weakness combined in some cases with cardiac conduction blocks, arrhythmias and congestive heart failure. Most patients show familial autosomal dominant or autosomal recessive patterns of inheritance, and a significant number of patients are sporadic. We report data on ten patients from four families and two sporadic cases of myopathy associated with pathogenic mutations in the desmin gene. The A337P mutation was identified in two siblings with late-onset skeletal myopathy and mild cardiac involvement. A heterozygous R406W mutation was present in a sporadic case with early-onset and relentlessly progressive skeletal and cardiac myopathy. Compound heterozygosity for two mutations, A360P and N393I, was detected in three siblings with extremely aggressive childhood-onset cardiomyopathy. Heterozygosity for N342D and I451M mutations was found in two families with relatively late onset of skeletal myopathy and no cardiac pathology. Finally, a splicing defect with deletion of the entire exon 3 was identified in a sporadic patient with slowly progressive cardiac and skeletal myopathy. None of these mutations was present in unrelated control individuals. Expression studies demonstrated inability of the mutated desmin to construct a filament network.

Program Nr: 574 from the 1999 ASHG Annual Meeting

Positional cloning of the gene responsible for Limb Girdle Muscular Dystrophy 1A. *M.A. Hauser[1], P. Salmikangas[2], S.K. Horrigan[3], K.D. Viles[1], U.M. Torian[1], U. Taivainen[2], L. Bartoloni[4], J.M. Stajich[1], P.C. Gaskell[1], J.R. Gilbert[1], C.A. Westbrook[4], J.M. Vance[1], M.A. Pericak-Vance[1], O. Carpén[2], M.C. Speer[1].* 1) Duke University Medical Center, Durham, NC; 2) University of Helsinki, Helsinki, Finland; 3) Georgetown University, Washington DC; 4) University of Illinois at Chicago, Chicago, IL.

We have positionally cloned the gene responsible for an autosomal dominant form of Limb Girdle Muscular Dystrophy (LGMD1A). We have previously reported a large North American family of German descent in which this adult onset disease is segregating. Symptoms include progressive weakness of the hip and shoulder girdles, as well as a distinctive dysarthric pattern of speech. Antibody staining shows that the dystrophin-associated glycoprotein complex is normal in muscle from these patients, indicating that the defect in LGMD1A involves a pathway that is entirely distinct from that of the autosomal recessive LGMD2 families. After linking LGMD1A to a region of about 2 megabases on 5q31, we identified a candidate gene that is expressed primarily in skeletal muscle. Analysis of cDNA sequence revealed this gene to be identical to myotilin, a novel sarcomeric protein that we have recently described. The amino terminus of myotilin is unique, whereas the carboxy teminus contains immunoglobulin-like domains that are homologous to titin. Myotilin is expressed in skeletal and cardiac muscle, and co-localizes with a-actinin. Yeast two-hybrid analysis indicates that the first 150 amino acids of myotilin interact with a-actinin. Initial characterization of this transcript has supported its involvement in the pathogenesis of LGMD1A. We have identified a C®T missense mutation in affected individuals that results in the conversion of threonine 57 to isoleucine. This change is present in all of the affected individuals that have been examined, yet has not been detected in 410 normal chromosomes. The effects of this alteration upon binding to a-actinin are under investigation. An additional base change in the 3' untranslated region also co-segregates with the disease. Mutation screening in additional unlinked autosomal dominant LGMD families is underway.

Program Nr: 575 from the 1999 ASHG Annual Meeting

Germline origins in the human factor IX gene: frequent somatic mosaicism with GC to AT. *R.P. Ketterling[1], J.B. Drost[2], X. Li[2], A. Mengos[2], C.K. Kasper[3], S.S. Sommer[2].* 1) Biochem/Molecular Biol, Mayo Clinic/Fdn, Rochester, MN; 2) Dept of Molecular Genetics, City of Hope Natl Med Cntr, Duarte, CA; 3) Orthopaedic Hospital, Los Angeles, CA.

The factor IX gene is advantageous for analyzing spontaneous germline mutations in humans. For 91 origin individuals (56 females and 35 males), the sequence of the leukocyte DNA was visualized directly by genomic sequencing for the presence or absence of the causative mutation. Somatic mosaicism was detected in 11 percent (7 females and 3 males) of these individuals with an estimated sensitivity of one mutant copy to 20 normal copies. Six of the ten defined somatic mosaics had GC to AT transitions at non-CpG dinucleotides. The preferentially embryonic occurrence of this *F9* mutation type over all other types (p=0.0024, Fishers Exact Test) may be responsible for its relatively more frequent observation as a causative mutation in the *F9* gene. In addition, since mutations occurring earlier in development have a higher probability of being inherited from the individual and, consequently, fixed in a population, this observation may help explain the AT mutational pressure (independent of CpG methylation-mediated transitions) which has maintained a GC content of 40 percent in the *F9* gene.

Program Nr: 576 from the 1999 ASHG Annual Meeting

Mutational analysis of a cohort Leber Congenital Amaurosis families. *R.A. Lewis[1], E.B. Abboud[2], A. Al-Rajhi[2], M. Leppert[3], J.R. Lupski[1], D.W. Stockton[1]*. 1) Baylor College of Medicine, Houston, TX; 2) King Khaled Eye Specialist Hospital, Kingdom of Saudi Arabia; 3) University of Utah, Salt Lake City, UT.

Leber Congenital Amaurosis (LCA) is known to be a clinically and genetically heterogeneous autosomal recessive retinal dystrophy and the most common genetic cause of congenital visual impairment in infants and children. Profound visual loss, characteristic behaviors, and markedly diminished electroretinograms typify the disorder. Mutations in two genes have been identified that cause recessive LCA, and a third gene has been associated with a sporadic or possibly dominant form. The first is in a retina-specific guanylate cyclase on chromosome 17p13, the second is the gene for a retinal pigment epithelium-specific protein, RPE65, on chromosome 1p31, and the third is the gene *CRX*, an OTX-like transcription factor on chromosome 19q13. Mutational screening of 45 multiplex families segregating recessive LCA and 4 families with a single affected member was evaluated at the three loci by direct DNA sequencing from intronic primers flanking the coding exons of each gene with standard techniques. Mutations were identified in only 8 (16%) of the families analyzed. The known heterogeneity of LCA is thus documented further. If these identified genes represent the most substantial contributions to this complex disease phenotype, we may expect more than 20 additional genes will be identified.

Program Nr: 577 from the 1999 ASHG Annual Meeting

Sjögren-Larsson syndrome: mutation analysis of the fatty aldehyde dehydrogenase gene in 60 probands. *Z. Lin, G. Carney, W.B. Rizzo.* Pediatrics and Human Genetics, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA.

Sjögren-Larsson syndrome (SLS) is an autosomal recessive disorder characterized by ichthyosis, mental retardation and spastic diplegia or tetraplegia. The disease is caused by mutations in the gene encoding fatty aldehyde dehydrogenase (FALDH), an enzyme that is necessary for oxidation of long-chain aliphatic aldehydes. To determine the genetic basis for SLS, we performed mutation analysis on 60 SLS probands from throughout the world. All patients were confirmed to have deficient FALDH enzyme activity in cultured skin fibroblasts. Mutations were detected by sequencing FALDH exons amplified by PCR from genomic DNA or by RT-PCR using fibroblast RNA. Among the SLS probands, 47 different mutations were detected including 10 deletions, 2 insertions, 21 missense mutations, 3 nonsense mutations, 3 complex deletion/substitutions, and 8 splice-site mutations. In addition, 4 single nucleotide polymorphisms (SNPs) (3 intronic and 1 exonic silent polymorphism) were found and their allele frequencies determined. Thirty-four of the mutations were private and the remaining mutations were carried by more than one proband. Haplotype analysis using the SNPs indicated that at least 3 of the common mutations (233G>A; 682C>T; 798+1delG) arose independently on multiple occasions. All of the splice-site mutations led to exon skipping or utilization of cryptic splice sites. The amino acid substitutions were distributed throughout the FALDH protein. Most of these mutations caused a profound reduction of enzyme catalytic activity when overexpressed in mammalian cells, but one mutation (798G>C) that altered the last nucleotide in exon 5 appeared to have a greater affect on mRNA stability. Our results indicate that SLS is caused by a variety of mutations, which will provide a framework for DNA diagnosis and understanding the correlation between structural and functional alterations in the enzyme.

Program Nr: 578 from the 1999 ASHG Annual Meeting

The *GJB3* gene also underlies non-syndromic recessive deafness. *X.Z. Liu[1,2], X.J. Xia[1], L.R. Xu[3], A. Pandya[1], C.Y. Liang[3], S. Blanton[1], K.P. Steel[4], S.D.M. Brown[5], W.E. Nance[1].* 1) Dept. of Human Genetics; 2) Dept. of Otolaryngolgoy, Medical Col of Virginia/VCU, Richmond, VA.USA; 3) Dept. of Otolaryngology, West China Univ of Medical Sciences, Chengdu, China; 4) MRC Institute of Hearing Research, Nottingham, UK; 5) MRC Mammalian Genetics Unit, Harwell, UK.

Mutations in the *GJB3* gene (Cx31) can cause a dominant non-syndromic form of hearing loss (DFNA2). To determine whether mutations at this locus can also cause non-syndromic recessive deafness, we screened 25 Chinese families with recessive deafness and identified affected individuals in two families who were compound heterozygous Cx31 mutations. The three affected individuals in the two families were born to non-consanguineous parents and had an early onset deafness. In the both families, differing SSCP patterns for the PCR of primer 3FR were observed in affected and unaffected individuals. Sequence analysis in the both families demonstrated an in-frame 3-bp deletion (423-5delATT) in one allele, which leads to the loss of an isoleucine residue at codon 141, and a 423A ® G transversion in the other allele, which creates an isoleucine-to-valine substitution at codon 141 (I141V). Neither of these two mutations was detected from 100 unrelated control subjects. The altered isoleucine residue lies within the third conserved a-helical transmembrane domain (M3), which is critical for the formation of the wall of the gap junction pore. This residue is conserved in mouse Cx31 and rat Cx31, whereas the other b-type connexins contains a phenylalanine at this position. Both the deletion of the isoleucine residue 141 and its substitution to valine in the two families could alter the structure of M3, and impair the function of the gap junction. We propose that mutations in families with dominant Cx31 mutations should have a dominant negative effect, since the normal hearing status of heterozygous carriers in our two recessive families provided no evidence that haploinsufficiency is a plausible alternative. The present data demonstrate that like Cx26, Cx31 can lead to both recessive and dominant non-syndromic forms of deafness. Work supported by the Jeffress Research Grant J-523, the Defeating Deafness, and NIHR01 grant-DC02530.

Program Nr: 579 from the 1999 ASHG Annual Meeting

Mutations in COL11A2 alter the structure of the tectorial membrane causing nonsyndromic hearing loss at the DFNA13 locus. *W.T. McGuirt[1], S.D. Prasad[1], A.J. Griffith[2], D.H. Kunst[3], K.B. Shpargel[2], R.F. Mueller[4], H.G. Brunner[3], C.W.R.J. Cremers[3], G.V. Camp[5], R.J.H. Smith[1].* 1) Molecular Otolaryngology Research Laboratories, Department of Otolaryngology, University of Iowa; 2) National Institute on Deafness and Other Communication Disorders, NIH; 3) Department of Otorhinolaryngology, University Hospital, Nijmegen The Netherlands; 4) Department of Clinical Genetics, St James's Hospital, Leeds, UK; 5) Department of Genetics, University of Antwerp, Belgium.

Inherited hearing impairment most often presents without other associated features and is referred to as non-syndromic hearing loss (NSHL). These losses are further classified by mode of inheritance (DFNA, dominant; DFNB, recessive; DFN, X-linked), using an integer suffix to specify hearing loss loci in order of discovery. To date, 26 autosomal dominant non-syndromic sensorineural hearing loss (ADNSHL) loci have been mapped (DFNA1-26) and nine ADNSHL genes have been cloned. This report describes mutations in a specific collagen gene, COL11A2, in two large families with ADNSHL at the DFNA13 locus. Mutations in COL11A2 have been associated with a spectrum of phenotypes that typically include midface hypoplasia, cleft palate, precocious arthritis and sensorineural hearing loss. The presence of isolated hearing loss in the described families represents the mildest phenotype associated with COL11A2 mutations. Additionally, we identified a COL11A2 mutation that produces a premature stop codon in an individual with a severe recessive form of otospondylomegaepiphyseal dysplasia (OSMED). Evaluation of the Col11a2 mouse mutant by auditory brainstem response testing demonstrates moderate-to-severe hearing loss. Col11a2 was localized to the otic capsule, spiral limbus, lateral wall of the cochlea, and saccular and utricular maculae by in situ hybridization. Histologic analysis of the cochlea in this mouse model revealed widely disorganized collagen fibrils in the tectorial membrane by electron microscopy. This result is consistent with the proposed role of COL11A2 in controlling interfibrillar spacing.

Program Nr: 580 from the 1999 ASHG Annual Meeting

A novel zinc finger gene in 8q24.1 is mutated in patients with tricho-rhino-phalangeal syndrome type I. *P. Momeni[1], G. Gloeckner[2], O. Schmidt[1], A. Rosenthal[2], B. Horsthemke[1], H.-J. Lüdecke[1]*. 1) Institut für Humangenetik, Universitätsklinikum, Essen, Germany; 2) Abteilung Genomanalyse, Institut für Molekulare Biotechnologie, Jena, Germany.

The tricho-rhino-phalangeal syndrome type I (TRPS I) is characterized by sparse scalp hair, pear-shaped nose, long philtrum, protruding ears, cone-shaped epiphysis and short stature. Based on chromosomal aberrations in patients with this disease, we mapped the affected gene to 8q24.1, centromeric to *EXT1*.

In order to identify the *TRPS* gene, we have constructed a PAC clone contig spanning several chromosomal breakpoints of TRPSI patients. Based on large scale genomic sequencing, exon prediction, cDNA cloning and exon-connecting RT-PCR, we have identified a gene which spans a translocation and an inversion breakpoint, respectively, in two patients with TRPSI. Northern blot analysis identified two transcripts of 7 and 11 kb in fetal tissues. The 7 kb transcript is encoded by six exons located within 200 kb of genomic DNA. It has an open reading frame of 3,843 bp, which code for 1,281 amino acids. Interestingly, the predicted protein sequence contains several zinc finger motifs of different types. A pair of C_2H_2 zinc fingers at the carboxy-terminus of the protein shows highest homology with a characteristic carboxy-terminal pair of zinc fingers in *IKAROS* transcription factor proteins. This domain mediates protein-protein interaction. Another zinc finger is of the C_2C_2-type, which shows significant homology to many GATA-binding transcription factors. In addition, there are six other domains with the potential of forming C_2H_2 zinc fingers of hitherto unknown function. By sequence analysis of the entire coding region in seven unrelated patients with apparently normal chromosomes, we identified four different mutations in three exons. Two are C to T transitions creating TGA stop codons. The other two mutations are an 1 and 4 bp insertion, respectively, which lead to premature stop codons. These findings strongly suggest that we have identified the *TRPS* gene. We are grateful to all physicians who referred patients to us, and to the Deutsche Forschungsgemeinschaft for financial support.

Program Nr: 581 from the 1999 ASHG Annual Meeting

Identification of a common Ile1173Phe mutation in the canalicular multispecific organic anion transporter gene in patients with Dubin-Johnson Syndrome of Iranian-Jewish origin. *R. Mor-Cohen, A. Zivelin, N. Rosenberg, U. Seligsohn.* Institute of Thrombosis and Hemostasis, Chaim Sheba Medical Center, Tel-Hashomer and Sackler Faculty of medicine , Tel-Aviv University, Israel.

Dubin-Johnson Syndrome (DJS) is an autosomal recessive genetic disorder manifested by conjugated hyperbilirubinemia and accumulation of a dark pigment in the liver. The disorder has recently been associated with mutations in the canalicular multispecific organic anion transporter (cMOAT) gene (Hepatology 25:1541, 1997; Am J Hum Genet 64:739, 1999). We used SSCP and DNA sequencing in search for mutations and polymorphisms in the cMOAT gene, and employed PCR and restriction analysis for their detection, in a previously described cluster of patients with DJS of Iranian-Jewish origin (Q J Med 39:549,1970). all 17 subjects examined (of 9 unrelated families) were homozygous for an Ile1173Phe mutation due to A®T subtitution in nucleotide 3517 in exon 25. The predicted change in the amino acid sequence is at the transmembrane domain of cMOAT. In the general Iranian-Jewish population heterozygosity for this mutation was observed in 5/133 subjects tested (3.8%), whereas none of 100 subjects of other Jewish ethnic groups carried the mutation. The following three polymorphisms in the cMOAT gene were identified and their frequency determined in the general Iranian-Jewish population: 1) 1-24(C®T) in the 5'UTR (6/78 alleles-7.7%); 2) Val417Ile in exon 10 (35/116 alleles-30%); 3) 4147-35(G®A) in intron 29 (10/120 alleles-8.3%). Analysis of the polymorphisms in the 17 DJS patients disclosed that all 34 alleles had the same haplotype (1-24C, 417Ile, 4147-35A). These data provide strong evidence for a novel mutation causing DJS and suggest a founder effect.

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Program Nr: 582 from the 1999 ASHG Annual Meeting

Splicing enhancer mutations that perturb alternative splicing: a new mechanism causing familial growth hormone deficiency. *C.T. Moseley[1], J.A. Phillips III[1], M.A. Prince[1], P.E. Mullis[2]*. 1) Department of Pediatrics, Division of Genetics, Vanderbilt University School of Medicine, Nashville, TN, USA; 2) Department of Pediatrics, Division of Endocrinology, University Children's Hospital, Inselspital, Bern, Switzerland.

Growth hormone deficiency (GHD) occurs in ~ 1/4000 births. We have found a series of dominant-negative human growth hormone (GH1) gene mutations that cause isolated GHD (IGHDII) because the mRNAs produced have exon 3 (E3) skipping, resulting in large amounts of only 17.5 kDa protein isoforms that are usually present in trace amounts. Normal GH1 splicing removes introns (IVSs) 1-4 to yield mRNA that contains exons 1-5 and encodes 22 kDa mature GH. Alternative GH1 splicing uses different combinations of splice sites and can yield mRNAs encoding 20, 17.5, 11.3, or 7.4 kDa isoforms. Previously we reported novel intron splicing enhancer (ISE) mutations in IVS3 that can also cause increased production of 17.5 kDa isoforms from E3 skipping that results in IGHDII. We report here our discovery of a GH1 exon splicing enhancer (ESE) found through studies of a natural mutation from an IGHDII individual. This mutation (E3+5 A®G) alters an ESE motif (GAAGAA®GAAGGA) which immediately follows the weak IVS2 3' splice site that must be used to produce 22 kDa GH. RT-PCR amplification of transcripts from an expression construct containing the ESE mutation yielded small amounts of the normal 22 but large amounts of 20 and 17.5 kDa mRNAs. Interestingly, the resulting 20 kDa isoforms arise from decreased use of the weak IVS2 3' splice site as well as increased use of a strong, cryptic 3' splice site in E3 following the ESE. Our data indicate that E3+5 A®G perturbs an ESE and that IGHDII may be caused by the increased production of both 20 and 17.5 rather than 17.5 kDa isoforms alone. Our findings are of general interest because they suggest that 1) both ESEs and ISEs regulate alternative splicing of GH1 transcripts, and mutations in either can cause IGHDII and 2) mutations of homologous splicing enhancers that regulate alternative splicing in other genes may cause a variety of genetic diseases.

Program Nr: 583 from the 1999 ASHG Annual Meeting

DGGE Analysis of the Tissue Non-Specific Alkaline Phosphatase Gene in Hypophosphatasia. *S.R. Mumm[1], J. Jones[1], P.S. Henthorn[2], M.C. Eddy[3], M.P. Whyte[1,3].* 1) Div Bone & Mineral Diseases, Washington Univ Sch Med, St Louis, MO; 2) School of Vet Med, Univ of Pennsylvania, Philadelphia, PA; 3) MRU, Shriners Hospital for Children, St Louis, MO.

Hypophosphatasia is a heritable form of rickets/osteomalacia for which there is no established medical treatment. This inborn error of metabolism manifests an extraordinary range of clinical severity spanning death in utero to premature loss of teeth. To delineate the molecular pathology causing the extreme clinical variability and various patterns of inheritance of hypophosphatasia, we initiated comprehensive mutational analysis of the tissue non-specific isoenzyme of alkaline phosphatase (TNSALP) gene in our large patient population. Genomic DNA from more than 120 probands, spanning the entire clinical spectrum, is being studied. Selecting initially 10 patients, single-strand conformational polymorphism (SSCP) analysis was used to detect mutations in the TNSALP gene. SSCP was performed using genomic DNA and primer sets that spanned each of the translated exons 2-12, including splice sites. We have detected approximately 70% of the potential mutations (assuming two mutations for each patient, and autosomal recessive inheritance). This success rate is in accord with published efficiencies for SSCP. To increase the efficiency of mutation detection, we are developing the technique of denaturing gradient gel electrophoresis (DGGE) for the TNSALP gene. Thus far, DGGE primers and conditions have been developed for exons 5, 10, and 12 (these exons contained the largest number of mutations as determined by the SSCP analysis). All of the known mutations (as determined by SSCP) have been detected by the DGGE analysis. In addition, new mutations were detected in exon 10 for two patients; these mutations were undetected by SSCP. Hence, our preliminary results suggest that the DGGE analysis will be more efficient than SSCP. The DGGE primers for the remaining exons are being developed and tested on the patient DNAs. Characterization of the TNSALP mutations in our large patient population will elucidate the molecular pathology and inheritance patterns, and should improve prognostication for hypophosphatasia.

Program Nr: 584 from the 1999 ASHG Annual Meeting

Correlation between hemochromatosis gene (*HFE*) mutations and mild iron overload expression. *C. Mura, G. Le Gac, O. Raguenes, AY. Mercier, C. Ferec.* Lab Biogenetique, CHU, UBO, Brest, France.

Hereditary hemochromatosis (HHC) is a common autosomal recessive genetic disorder of iron metabolism characterized by increased intestinal iron absorption. HFE gene, encoding a protein which seems to play a role in iron uptake by interacting with the transferrin receptor, is clearly involved in HHC. Two main mutations have been described, C282Y and H63D substitutions accounting for approximately 90% of the HC chromosomes. We previously reported on the increase of the S65C variant in HC chromosomes neither carrier of C282Y nor H63D. In addition, C282Y homozygotes were clearly associated with a more severe form of the disease than individuals carrying other genotypes. A series of 708 patients with various symptoms which could suggest an iron overload but without reaching clinical HC criteria was analysed for HFE mutations and a phenotype-genotype correlation was established. Most of the patients were selected because of an iron marker value above the threshold defined for iron overloading, among them 67% showed a serum ferritin above the threshold. We identified 45% of carrier chromosomes in the studied group of patients. In addition, significantly higher frequencies of compound C282Y/H63D heterozygous and H63D/H63D homozygous genotypes were found than in HC probands and controls; whereas compound C282Y/S65C or H63D/S65C heterozygotes were not significantly increased. The iron marker values, i.e. transferrin saturation percentage, serum ferritin and serum iron concentrations were significantly higher in each group of patients who carried at least one HFE mutation compared with controls. Thus, these patients seem to constitute an intermediate phenotypic group that shows mild iron overload, and compound heterozygotes and homozygotes, as also found in HC probands, present a potential risk of developing complications that may have implication in genetic counseling.

Program Nr: 585 from the 1999 ASHG Annual Meeting

Gene dosage of a 6p25 gene causes defects of the anterior chamber of the eye: FKHL7 or HFH1? *D.Y. Nishimura[1], C.C. Searby[1], D.S. Walton[2], S.R. Patil[1], A.B. Kanis[1], E.M. Stone[1], W.L.M. Alward[1], V.C. Sheffield[1]*. 1) University of Iowa, Iowa City, IA; 2) Harvard Medical School, Boston, MA.

We recently used positional cloning methods to identify the human transcription factor gene FKHL7 as a cause of glaucoma resulting from developmental defects of the anterior chamber of the eye. Some reported mutations suggest a haploinsufficiency model of disease development. We have now identified patients with 6p25 duplications and anterior chamber defects of the eye. Molecular techniques were used to characterize the extent of the duplications within the 6p25 region. Among the cases identified are four members of a family with congenital iris hypoplasia and a microduplication involving 6p25. In addition, we identified a single proband with Peters anomaly and a 6p25 duplication. The association of these two independent duplication events with developmental defects of the anterior chamber of the eye indicates that dosage of a gene or genes within this interval is important for normal development of the eye to occur. The FKHL7 transcription factor is located within the duplicated region and is thus a strong candidate for being one of these genes. However, the duplications also encompass a region on 6p25 for which a second locus involved in eye development has been postulated. We have analyzed a second forkhead transcription factor gene, Hfh1 as a possible candidate gene for this second locus. This gene is also contained within the duplicated region. No Hfh1 mutations were found in probands with anterior chamber defects although novel FKHL7 mutations have been found in these patients, providing additional support for the involvement of FKHL7 mutations in causing anterior chamber defects. The results of this study demonstrate that linked families with no apparent mutations in FKHL7 should be carefully screened for duplications or deletions of the entire FKHL7 gene.

Program Nr: 586 from the 1999 ASHG Annual Meeting

Dysferlin and the dystrophin-associated proteins. *F. Piccolo, D.P. Venzke, K.P. Campbell.* Howard Hughes Medical Inst., University of Iowa, Iowa City, IA.

Recently, the dysferlin gene has been cloned and mutations in it have been described in both MM (Miyoshy Myopathy) and LGMD2B (autosomal recessive Limb Girdle Muscular Dystrophy type B) patients (Bashir et al. Nature Gen., 1999; Vol.20, 37-42 and Liu et al. Nature Gen., 1999; Vol.20, 31-36). Dysferlin has been predicted to be homologous to the fertilization factor Fer-1 of C. elegans but the role of this protein in mammalians remains unknown. To assess the function of dysferlin in skeletal muscle, we first developed a mouse monoclonal and two rabbit polyclonal antibodies. Here we demonstrate by immunofluorescence (IF) and western blot that dysferlin is a membrane protein present at the sarcolemma. Because of its membrane localization and because sarcoglycans (which genes are responsible for LGMD2D (alpha-SG), 2E (beta-SG), 2C (gamma-SG), and 2F (delta-SG)) are components of the dystrophin glycoprotein complex (DGC), we hypothesize that dysferlin could be a new member of the dystrophin associated protein family. To test this hypothesis, we analyzed by IF a large cohort of Duchenne/Becker Muscular Dystrophy (D/BMD) and LGMD2 muscle biopsies from sporadic cases (sarcoglycanopathies and non-sarcoglycanopathies). Interestingly, we found that 20 percent of D/BMD and 80 percent of the sarcoglycanopathies present a secondary deficiency of dysferlin, indicating a relation between dysferlin and the DGC. To better understand the nature of this relationship, we isolated the DGC from rabbit skeletal muscle and tested for the presence of dysferlin by western blot. Preliminary data indicate that dysferlin is a structural component of the sarcolemma but it is not an integral component of the DGC. We were also able to estimate that 50 percent of the LGMD2 non sarcoglycanopathies present a deficiency of dysferlin at the sarcolemma. These results suggest that dysferlinopathies are extremely frequent among LGMD patients and a screening with antibodies against dysferlin is highly recommended for diagnostic purposes.

Program Nr: 587 from the 1999 ASHG Annual Meeting

KCNE1 like gene id deleted in AMME contiguous gene syndrome: identification and characterization of the human and mouse homologs. *A. Renieri[1], F. Vitelli[1], M. Piccini[1], I. Meloni[1], L.J. Galietta[2], O. Moran[3], A. Bulfone[4], S. Banfi[4], B. Pober[5].* 1) Dept Molec Biol, Medical Genetics, University of Siena, Siena, Italy; 2) Genetica Molecolare, Istituto G. Gaslini Genova, Italy; 3) Istituto di Cibernetica e Biofisica del CNR, Genova; 4) Telethon Institute of Genetics and Medicine (TIGEM), 20132 Milan, Italy; 5) Dept. Genet., Yale University, School of Medicine, New Haven CT.

We describe the identification and characterization of a new gene deleted in the AMME contiguous gene syndrome. This gene is predominantly expressed in heart, skeletal muscle, spinal cord and brain. Screening of placenta and NT2 cDNA libraries enabled us to obtain the 1.5 Kb full length transcript which shows a 426bp open reading frame. Since, the resulting 142 amino acids peptide has a single putative transmembrane domain and a weak but suggestive homology with KCNE1 (minK), a protein associated with KCNQ1 potassium channel (KVLQT1), we named this new gene KCNE1 Like (KCNE1L). To get more insight into this new member of an apparently distinct protein family, we have identified and characterized the homologous mouse gene (Kcne1l), which encodes a peptide of 143 amino acids with 91% homology and 80% identity. Expression pattern of mouse Kcne1l in the developing embryo revealed strong signal in ganglia, in the migrating neural crest cells of cranial nerves, in the somites and in the myoepicardial layer of the heart. We are currently exploring the possibility that the expression of KCNE1L could modulate the currents expressed by KCNQ1-3 genes, in oocytes of Xenopus laevis. The specific distribution in adult tissues, the putative channel function and the expression pattern in the developing mouse embryo suggests that KCNE1L could be involved in the development of the cardiac abnormalities, as well as of some neurological signs observed in the patients with AMME contiguous gene syndrome.

Program Nr: 588 from the 1999 ASHG Annual Meeting

Isolation of a novel motor-like protein that interacts with Retinitis Pigmentosa GTPase Regulator (RPGR) in the rod outer segments of the retina. *R. Roepman[1], N. Bernoud-Hubac[2], D. Schick[2], A. Maugeri[1], H.H. Ropers[1,3], W. Berger[3], F. Cremers[1], P. Ferreira[2].* 1) Dept. of Human Genetics, University Hospital Nijmegen, Nijmegen, The Netherlands; 2) Dept. of Pharmacology, Medical College of Wisconsin, Milwaukee, WI; 3) Max-Planck Institute of Molecular Genetics, Berlin, Germany.

The Retinitis Pigmentosa GTPase Regulator (*RPGR*) gene that is involved in X-linked retinitis pigmentosa type 3 (RP3) is ubiquitously expressed, in contrast to the disease phenotype that is restricted to the neural retina layers of the eye. The function of RPGR is not known, though its N-terminal half consists of a RCC1-like domain (RLD) in which most RP3 mutations, including all of the missense mutations, were found. RCC1 is a guanine-nucleotide exchange factor (GEF) of the Ras-like GTPase, Ran. Ras-related proteins and their modulators are often key mediators of protein trafficking in a vectorial fashion. Our goals are to understand the role of normal and mutated RPGR, respectively, in retinal function and pathogenesis of RP3. The RLD of RPGR was used in a yeast two-hybrid screen of two bovine retina cDNA libraries to identify substrates that interact with RPGR in the retina. We identified a novel, RPGR-binding protein (RPGR-BP) that shows preferential expression in the retina. The human orthologue has been isolated and interaction with RPGR has been confirmed. Chromosomal mapping positioned the corresponding gene at 14q11. RPGR-BP has no striking homology to any protein with a known function, however analysis of its primary structure suggests that it is a novel motor protein. Immunohistochemical studies of RPGR and RPGR-BP revealed a co-localization in the rod outer segment layer of the retina. The motor protein-like structure of RPGR-BP and its localization in the rod outer segments hints towards an involvement in rod photoreceptor-specific membrane transport processes. Studies towards the exact nature of this involvement are underway.

Program Nr: 589 from the 1999 ASHG Annual Meeting

Is guanylate cyclase activating protein 3 (GCAP3) the fourth gene for Leber's congenital amaurosis ? *J.-M. Rozet[1], I. Perrault[1], S. Gerber[1], D. Ducroq[1], I. Ghazi[2], J.-L. Dufier[2], A. Munnich[1], J. Kaplan[1].* 1) Unité de Recherches sur les Handicaps Génétiques de l'Enfant, INSERM U393, Hôpital Necker, Paris, France; 2) Service d'Ophtalmologie, Hôpital Necker, Paris, France.

The retinal-specific guanylate cyclase gene (retGC1) which accounts for about 1/5 cases of Leber's congenital amaurosis (LCA), represents the major disease causing gene in LCA. So far, three guanylate cyclase activating proteins (GCAP) have been identified. All three forms were found to stimulate retGC1 in low $[Ca^{2+}]_{free}$ leading to restoration of cGMP concentration after hyperpolarization of the plasma membrane following the photo-excitation in the retina. Defects in GCAPs are expected to result in the inability of photoreceptor cells to restore the cGMP level, a situation functionally equivalent to defects in the retGC1 gene. Thus, GCAPs were considered as excellent candidate genes for LCA. So far, GCAP1 and GCAP2 have been excluded as LCA causing genes. We report here the study of the GCAP3 gene in 84 patients affected with LCA. All four exons as well as the intron-exon boundaries were screened for mutations using SSCP and direct sequencing. Five different nucleotide substitutions were identified in four families and were absent in 80 controls. One of these changes alters an amino acid in the EF2 motif which is highly conserved across GCAPs and in the calmodulin gene. Two other changes alter an amino acid conserved in GCAP1. Interestingly, one patient was found to harbor mutations in each of GCAP3 alleles, inherited from his healthy parents, raising the question of whether GCAP3 could be the fourth gene responsible for LCA.

Program Nr: 590 from the 1999 ASHG Annual Meeting

Characterization of the *NPHP1* locus: Mutational mechanism involved in the large deletion observed in familial juvenile nephronophthisis patients. *S. Saunier[1], F. Blanchard[2], J. Calado[2], J. Weissenbach[3], C. Antignac[1]*. 1) Division Signal Transduction, Beth Israel Deaconee MC, HIM, Boston, MA; 2) INSERM U423, Hopital Necker, Paris, France; 3) CNS, Evry, France.

Familial juvenile nephronophthisis (NPH) is an autosomal recessive, genetically heterogeneous, kidney disorder, representing the most frequent inherited cause of chronic renal failure in children. A gene, *NPHP1*, responsible for approximately 85% of the purely renal form of nephronophthisis, was identified by a positional cloning strategy. The major *NPHP1* gene defect is a large homozygous deletion of ~250 kb found in about 80% of the patients. In this study, by large scale genomic sequencing and pulse field gel analysis, we have characterized the complex organization of the *NPHP1* locus and determined a mutational mechanism which results in the large deletion observed in most NPH patients. We showed that *NPHP1* is flanked by two large inverted repeats of ~330 kb (proximal copy-330RI, distal copy-330RII). In addition, a second sequence of 45 kb (45RI) located adjacent to 330RI repeat was shown to be directly repeated 250 kb away within 330RII repeat abolishing the STS 804H10R present in 330RI. The patients deletion breakpoint is located within the 45 kb repeat, suggesting an unequal recombination between the two homologous copies of the smaller repeat. This event could be the result of chromosomal misalignment followed by unequal cross-over or by the formation of a loop structure on a single chromosome, leading to the 250 kb deletion observed in the patients. Moreover, we demonstrated a non pathologic rearrangement involving the two 330 kb inverted repeats found in 11 patients, and also observed in 1,2% of control individuals in the homozygous state. This rearrangement could be explained by a mispairing of the 330 kb inverted repeat followed by double recombination, leading to the complete absence of 804H10R and the presence of another copy of the 45RII repeat. This complex rearrangement, as well as the common deletion found in most of NPH patients, illustrate the high level of rearrangements occuring in the centromeric region of chromosome 2.

Program Nr: 591 from the 1999 ASHG Annual Meeting

New mutations in collagen VIa**1,** a**2 genes cause autosomal dominant muscular dystrophy.** *P.C. Scacheri[1,5], E.M. Gillanders[2], S. Subramony[3], V. Vedanarayayanan[3], C.A. Crowe[4], M. Bingler[1], E.P. Hoffman[5].* 1) Biochem. & Molecular Genetics, University of Pittsburgh, Pittsburgh, PA; 2) NIH-NHGRI, Bethesda, MD; 3) Unversity of Mississippi Med Ctr, Jackson, MS; 4) MetroHealth Med Ctr, Cleveland, OH; 5) Children's Nat'l Med Ctr, Washington DC.

We collected 3 large families diagnosed with autosomal dominant limb-girdle muscular dystrophy. Both intra- and interfamilial variability was seen; symptoms ranged from loss of ambulation to a relatively mild myopathy. Muscle biopsy findings include marked fatty infiltration and increased connective tissue, with evidence of fiber degeneration/regeneration and variable fiber size. Through genome-wide linkage studies, we linked these 3 families to the Bethlem myopathy locus on chromosome 21q (Zmax 7.68, q=0.05). Bethlem myopathy is defined as a benign congenital myopathy with joint contractures caused by mutations in collagen VI genes. Despite phenotypic overlap, our patients have a more dystrophic phenotype than typically seen in Bethlem myopathy. Most of the Bethlem mutations reported to date are dominant negative, and involve glycine substitutions in the triple helical domains of collagen VIa1 and a2. We identified the causative mutations in all three of our families using SSCP analysis and direct sequencing of genomic DNA. Family 1 has a glycine to aspartic acid substitution in the triple helical domain of collagen VIa1 (G341D). Family 2 has a lysine to arginine substitution in the amino globular domain of collagen VIa1 (K121R). Family 3 has an aspartic acid to asparagine substitution in the carboxy globular domain of collagen VIa2 (D354N). All affected patients were heterozygous for the mutations, and base substitutions were not found in 95 unrelated individuals. Mutations in the globular domains of colVIa1 or colVIa2 are not previously reported, and may account for the more dystrophic phenotype in our families. We hypothesize that these collagen VI mutations disrupt the assembly of multimeric collagen VI proteins, which ultimately disturb protein/protein interactions in the basal lamina and lead to an abnormal development or regeneration of muscle.

Program Nr: 592 from the 1999 ASHG Annual Meeting

Unsuspected Saethre-Chotzen syndrome patients with insertions and deletions in a normally mutant-free region of the TWIST gene. *F.V. Schaefer, C.A. Shults, C.M. Outlaw.* Molecular Genetics, HA Chapman Inst Medical Gen, Tulsa, OK.

Apert, Crouzon, Jackson-Weiss or Pfeiffer syndromes have been unified by the discovery that most are the result of different mutations in common regions of the FGFR1,2&3 genes. However, 25% of patients do not have a mutation within the tested regions of these genes. We examined the hypothesis that a portion of the patients have unrecognized Saethre-Chotzen syndrome with mutations in TWIST.

The TWIST gene is composed of a coding region that involves a DNA-binding region followed by a helix-loop-helix (HLH) motif characteristic of transcriptional regulators. All Saethre-Chotzen syndrome patients that have been published have either a mutation in the DNA-binding-HLH motif, a stop mutation that eliminates this structure or a deletion of the entire gene. A panel of craniosynostosis patients that had proved not to have the predicted mutations in the FGFR genes were tested for alterations in the TWIST gene by PCR amplification, gel electrophoresis and DNA sequencing.

The results of the testing showed (1) about 20% (4/20) were found to have a detectable mutation in the TWIST gene. The mutations included a 3bp insertion, a 21bp insertion, an 18bp deletion and a gly to ser point mutation. All these gene alterations were in phase and none resulted in protein truncation. (2) Strikingly, **all** these mutations were located in the coding upstream of the DNA-binding-HLH region where no previous mutations causing classical Saethre-Chotzen syndrome had been described.

These results lead to the conclusions that (1) there are mutations causing Saethre-Chotzen syndrome in a region of the gene not previously suspected to be integrally involved in gene function or pathology. (2) These mutations may lead to unique symptomology recognized to be craniostenosis but not classical Saethre-Chotzen syndrome. (3) Saethre-Chotzen patients with mutations in the TWIST gene do appear to contribute more significantly than previously suspected to the over all craniosynostosis population. Consequently, patients should be examined for TWIST as well as FGFR mutations.

Program Nr: 593 from the 1999 ASHG Annual Meeting

Mutations that result in premature termination codons in the COL5A1 gene of type V collagen produce Ehlers-Danlos syndrome (EDS) type I or II. *U. Schwarze[1], M. Atkinson[1], D.S. Greenspan[2], P.H. Byers[1].* 1) University of Washington, Seattle, WA; 2) University of Wisconsin, Madison, WI.

EDS types I and II are characterized by skin hyperextensibility and fragility, and joint hypermobility. Linkage studies suggest that most affected individuals have mutations in either the COL5A1 or COL5A2 genes that encode the chains of type V collagen, a ubiquitous protein associated with large fibrils in the extracellular matrix. Despite these findings only a small proportion of mutations have been identified by examination of cDNA synthesized from RNA derived from cells of affected individuals. To try to identify the remaining classes of mutations, we studied cells from 16 individuals with EDS type I or II. We isolated mRNA from their cultured dermal fibroblasts, screened large overlapping fragments of the COL5A1 and COL5A2 cDNA by polyacrylamide gel electrophoresis to identify insertion/deletion mutations, and then used expressed polymorphic sequences in the COL5A1 gene to determine if both alleles were expressed. In cells from 2 individuals there were splice site mutations in different regions of the COL5A1 gene (IVS4-2AG and IVS14-11TA) both of which resulted in complex splice abnormalities. Of the remaining 14, 13 were heterozygous for at least one of 4 coding sequence polymorphisms in COL5A1 and, of these, 6 had stable mRNA from only one allele. We screened 66 exons of COL5A1 genomic DNA by electrophoresis of amplified exons on heteroduplex gels and sequenced variant fragments. We identified the causative mutations in 4 of these individuals. In exon 49 of the 66 exon gene 3900CinsC results in a downstream stop in exon 50, 3886delC in exon 49 and 4147delC produce the same stop in exon 58, and 4201GinsA produces a stop in exon 57. In each instance sequence of the stable mRNA identified only the normal allele. These results suggest that more than a third of affected individuals have mutations that lead to premature termination codons in one COL5A1 allele which are destabilized by the pathway of nonsense mediated decay. These result in production of half the normal amount of type V collagen, alter fibril formation, and lead to marked biomechanical abnormalities. (AR21557).

Program Nr: 594 from the 1999 ASHG Annual Meeting

Are genes contiguous to glucocerebrosidase involved in patients with Gaucher disease who have parkinsonian symptoms? *E. Sidransky[1], N. Tayebi[1], M. Callahan[1], D. Stone[1], V. Madike[1], J.J. Filiano[2], B. Bembi[3], A. Tylki-Szymanska[4], E.I. Ginns[1]*. 1) DHHS/PHS, NSB/NIMH/NIH, Bethesda, MD; 2) Dept of Pediatric Neurology, Children's Hosp at Dartmouth, Lebanon, NH; 3) Gaucher Center, Trieste, Italy; 4) Children's Memorial Health Inst, Warsaw, Poland.

There have been several reports of adult patients with Gaucher disease, the inherited deficiency of lysosomal glucocerebrosidase (GC), and parkinsonian symptoms. We evaluated a female patient diagnosed with Gaucher disease at age 19 because of mild anemia. An elective splenectomy was performed at age 34. She developed a tremor at age 42 and rapid deterioration of her gait followed. A pallidotomy performed at age 47 resulted in no significant improvement. On examination she had impaired horizontal saccadic eye movements, but no hematologic or skeletal abnormalities. Neurologic symptoms were not improved with enzyme replacement, but she showed some response to L-dopa. Sequencing of the synuclein gene failed to identify any mutations. Sequencing of the 11 exonic regions of the GC gene revealed the mutation L444P on the paternal allele and D409H on the maternal allele. Southern analyses showed the maternal allele had an additional 15 kb fragment resulting from a recombination between metaxin and its pseudogene. Metaxin, a convergently transcribed gene located adjacent to the GC pseudogene, encodes for a 317 aa protein which is part of a preprotein import complex in the outer membrane of the mammalian mitochondrion. Sequencing of the extra fragment confirmed a duplication carrying a fusion gene. Analysis of a highly polymorphic tetranucleotide (AAAT) repeat located 3' to the GC gene revealed 3 allelic forms, suggesting a duplication of the region encompassing the GC pseudogene and metaxin. Three additional patients with Gaucher disease and parkinsonian symptoms have been identified. Sequencing has revealed that there is no shared genotype among these parkinsonian patients, whose genotypes are N370S/IVS2+1, N370S/recombinant allele, and G377S/G377S. Further analyses of the GC locus in these patients are being performed to explore the possible association of the parkinsonian symptoms and alterations in contiguous genes.

Program Nr: 595 from the 1999 ASHG Annual Meeting

Disturbed interaction between Huntingtin and HIP3 implicates defects in neuronal endocytosis in the pathogenesis of HD. *R.R. Singaraja[1], S. Hadano[2], M. Metzler[1], C. Wellington[1], B. Leavitt[1], W. Balch[3], S. Bannykh[3], K. Fichter[1], A. Hackam[1], L. Gan[1], T. Zhang[1], A. Yassa[1], K. McCutcheon[1], J.M.L. Michel[1], V. Chopra[1], S. Sherer[4], D. Geitz[5], M.R. Hayden[1].* 1) Medical Genetics/CMMT, University of British Columbia, Vancouver, British Columbi, Canada; 2) Tokai University School of Medicine, Kanagawa , Japan; 3) Scripps Research Institute, La Jolla, California, USA; 4) Dept of Gen., HSC, Toronto, ON, Canada; 5) Dept of Human Genetics, University of Manitoba, Winnipeg, MAN, Canada.

Huntington Disease (HD) is caused by an expansion of a polyglutamine tract in the coding region of a novel gene. Despite it's cloning approximately 6 years ago, the normal function of huntingtin is still unclear. We have isolated a novel huntingtin interacting protein, termed HIP3, utilizing the yeast two hybrid system. The HIP3 protein contains several ankyrin repeats and shows striking similarity (38%) to the yeast protein Akr1which is essential for endocytosis in S. cerevisiae. The HIP3 protein is expressed predominantly in the brain, and is found in neurons of regions including the cortex, striatum and hippocampus, all areas affected in Huntington disease. Deconvolution confocal microscopy revealed HIP3 immunoreactivity in structures associated with the Golgi complex. HIP3 shows decreased interaction with mutant huntingtin, as measured by semi-quantitative b-galactosidase assays and by in-vitro binding assays. This data, together with the fact that the HIP1 homologue in yeast, Sla2, has an essential role in endocytosis, indicates a role for wild type huntingtin in the vesicle transport pathways of neurons, and shows that the pathogenesis of HD is associated with disturbed interactions with proteins essential for endocytosis in neurons.

Program Nr: 596 from the 1999 ASHG Annual Meeting

Human aryl-hydrocarbon interacting protein-like 1 gene (*AIPL1*), a candidate for inherited retinal disorders: mapping to 17p13, characterization and mutation testing. *M.M. Sohocki[1], S. Blackshaw[2], C.L. Cepko[2], L.S. Sullivan[1,3], S.P. Daiger[1,3]*. 1) Human Genetics Center, School of Public Health, University of Texas HSC, Houston, TX; 2) Dept. of Genetics and Howard Hughes Medical Institute, Harvard Medical School, Boston, MA; 3) Dept. of Ophthalmology and Visual Science, University of Texas HSC, Houston, TX.

In a previous study, we mapped retina/pineal-expressed EST (expressed sequence tag) clusters as candidate genes for inherited retinal disorders(Genomics 58:29-33,1999). Two EST clusters, THC220430 and THC90422, map to 17p13. Several loci causing inherited retinal degeneration map to this region, including autosomal dominant cone-rod dystrophy (CORD5), recessive cone-rod dystrophy and central areolar choroidal dystrophy.

In the current study, we determined that the cDNAs of these two EST clusters overlap and represent coding sequences of a single gene. This gene has high sequence similarity to human aryl hydrocarbon-interacting protein (*AIP*), a member of the FKBP family, and has been named aryl hydrocarbon-interacting protein-like-1 (*AIPL1*). AIP has been implicated as a molecular chaperone and/or nuclear targeting factor in Ah-mediated signaling; therefore, it is possible that AIPL1 performs a similar nuclear transport or protein folding function in the retina and pineal gland. The genomic structure of *AIPL1* was characterized; the gene contains four exons encoding a protein 384 amino acids in length, with a molecular mass of 43,865 daltons, and a deduced pI of 5.56. In addition, *in situ* hybridization of AIPL1 indicated a very high photoreceptor-specific expression, strengthening *AIPL1* as a candidate gene for retinal degeneration.

We are currently screening for mutations in patient samples from families with inherited retinal diseases mapping to the *AIPL1* region of 17p13.

Supported by grants from the Foundation Fighting Blindness, the George Gund Foundation, by the William Stamps Farish Fund and the M.D. Anderson Foundation, by NIH Grant EY07142, and by NIH-NEI National Institutional Service Award EY07024.

Program Nr: 597 from the 1999 ASHG Annual Meeting

Organization and mutations of the human Chediak-Higashi syndrome gene (*CHS1*) in the childhood and adult forms of the disorder. *R.A. Spritz[1], M.A. Karim[1], K. Suzuki[1], D.L. Nagle[2], K.J. Moore[2]*. 1) Human Medical Genetics Program, Univ Colorado Health Sci Ctr, Denver, CO; 2) Millennium Pharmaceuticals, Inc., Cambridge, MA.

Chediak-Higashi syndrome (CHS) is an autosomal recessive disorder characterized by hypopigmentation, bleeding tendency, and severe immunodeficiency. Most patients present in childhood with recurrent bacterial infections due to neutropenia and lack of natural killer (NK) cell activity. About 85-90 percent of CHS patients develop an accelerated lymphoproliferative phase that is fatal unless treated by bone marrow transplantation. However, about 10-15 percent of patients have few or no severe infections, develop no accelerated phase, and survive to adulthood. Patients with such adult CHS develop progressive neurologic disease that is debilitating and often fatal. We previously described the cloning of the human gene and mutations of the *CHS1* gene in patients with severe childhood CHS. All of these patients, as well as those in mice with the homologous disorder, *beige*, have "protein-null" mutations: frameshifts, nonsense mutations, or deletions that would abolish expression of full-length CHS1 polypeptide. We have now defined the exon-intron organization of the human *CHS1* gene, which consists of 54 exons distributed over >100 kb, and we have used this information to search for *CHS1* gene mutations in 18 new patients with CHS. We identified a number of novel frameshift and nonsense mutations, particularly in patients with the severe childhood form of CHS. Of greater interest, we identified missense mutations in patients with the clinically milder "adult" form of the disorder. Our results establish that the classic childhood and the milder adult forms of CHS are allelic, resulting from protein-null and missense mutations of the *CHS1* gene, respectively. In several patients with the clinical diagnosis of Chediak-Higashi syndrome we found no mutations of the *CHS1* gene. Genetic analysis in some of these families appeared to exclude the 1q43 region, suggesting the possibility of locus heterogeneity for the Chediak-Higashi syndrome in man.

Program Nr: 598 from the 1999 ASHG Annual Meeting

Werner protein (WRN) interacts with a subunit of DNA polymerase δ, an essential enzyme in DNA replication.
A.M. Szekely, Y-H. Chen, C-Y. Zhang, S.M. Weissman. Department of Genetics, Yale University Sch Medicine, New Haven, CT.

Werner syndrome (WS) is a rare autosomal recessive disorder with many features of premature aging. The clinical and cellular phenotypes of WS show remarkable similarity to normal human aging. The defective gene for WS (WRN) displays significant homology to the RecQ family of DNA helicases, however its precise function is largely unknown.

To obtain insights into the function of WRN gene, we screened for proteins that specifically interact with the WRN gene product. Using the yeast two-hybrid system we isolated p50, a key subunit of the human DNA polymerase δ enzyme complex essential in DNA replication and implicated in DNA repair. The interaction between the WRNp and p50 is specific, mediated by the C terminus portion of WRNp (WRNCTp) and occurs both in vitro and in vivo. In mammalian cells we found that WRNp predominantly localizes to the nucleolus via its C terminus domain, while p50 is detected exclusively in the nucleoplasm. Intriguingly, in co-transfection experiments WRNp recruits p50 into the nucleolus upon their in vivo interaction. Further analysis of components of this protein complex is under way. Moreover, novel candidate proteins, some without homology to known genes, were also isolated by their virtue of specific interaction with WRNCTp.

Our data indicate that WRNp can directly associate with other proteins both in vitro and in vivo. The interaction between WRNp and a subunit of DNA polymerase δ may provide the molecular link to the findings that replication initiation is impaired in WS cells. Moreover, our data suggest that WRNp may be part of multiple protein complexes with dynamic changes in their composition and subcellular/subnuclear localization. Unraveling the precise function of WRN gene may provide insights into molecular mechanisms that underlie the rate and variability of human aging including susceptibility to age-related disorders.

Program Nr: 599 from the 1999 ASHG Annual Meeting

Filamin 2, a Muscle-Specific Form of Filamin, Interacts with g-sarcoglycan, a Member of the Dystrohin Glycoprotein Complex. *T.G. Thompson[1], S. Watkins[2], Y. Chan[1], M. Speer[3], M. Brosius[1], J. Vance[3], A. Hack[4], E. McNally[4], H. Lidov[1], M.P. Vance[3], L. Kunkel[1]*. 1) Department of Medicine, Genetics division, Children's Hospital, Boston, MA; 2) Center for Biological Imaging, University of Pittsburgh, Pittsburgh, PA; 3) Department of Medicine, Duke University Medical Center, Durham, NC; 4) Department of Medicine, Section of Cardiology, University of Chicago, Chicago, IL.

Mutations in genes encoding for the sarcoglycans, a subset of proteins within the dystrophin glycoprotein complex (DGC), produce a limb-girdle muscular dystrophy phenotype; however, the precise role of this group of proteins in skeletal muscle is not known. In order to better understand the role of the sarcoglycan complex, we looked for sarcoglycan interacting proteins with the hope of finding novel members of the DGC. Using the yeast two-hybrid method, we have identified a skeletal muscle-specific form of filamin, which we term filamin 2 (FLN2), as a g-sarcoglycan interacting protein. Previous studies of filamin family members have determined that these proteins are involved in actin reorganization associated with cell migration, adhesion, force transduction, differentiation, and survival. The predicted FLN2 protein is 74% identical to FLN1, and, by northern and western analysis, FLN2 appears to be expressed in only the skeletal and cardiac muscles. In addition, using immunoglold electron microscopy and confocal immunofluorescence, we determined that FLN2 is located at the sarcolemmal membrane and within the contractile apparatus, suggesting that FLN2 accomplishes comparable tasks in muscle cells as FLN1 in nonmuscle cells. Interestingly, we have localized FLN2 to a region overlapping with the LGMD1E region on chromosome 7 and are currently searching for FLN2 mutations in LGMD1E families. The finding of FLN2 as a sarcoglycan complex member introduces new implications for the pathogenesis of muscular dystrophy.

Program Nr: 600 from the 1999 ASHG Annual Meeting

Molecular Pathology of the SMN and H4F5 genes in Spanish SMA patients. *E.F. Tizzano[1], Y. Martin[2], BM.J. Barcelo[1], I. Cusc[1], M. Cornet[1], E. Bussaglia[1], A. Valero[2], C. Hernandez- Chico[2], C. Soler[1], M. Baiget[1].* 1) Genetics, Hospital Sant Pau, Barcelona, Barcelona, Spain; 2) Genetics, Hospital Ramon y Cajal, Madrid, Spain.

Spinal muscular atrophy (SMA) is classified by age of onset and maximal motor milestones achieved in type I (severe form), type II (intermediate form) and type III (mild/moderate form). We have analyzed 364 families (169 type I, 119 type II and 79 type III) looking for mutations in the SMA determining gene SMN (Survival Motor Neuron) located at 5q31. Three hundred and eight patients (85%)have deletions of the exon 7 and 8 of the SMN telomeric gene; in 18 patients(5%) centromeric-telomeric hybrid genes were detected; 7 cases (2%) showed a 4bp deletion in exon 3 (delAGAG) and in one type II case, an 11 bp duplication in the exon 6 was detected. In the remaining 28 patients (7%), no mutation so far was identified. All types of mutations described were associated with the three different phenotypes. The delAGAG of exon 3 was detected in one type I case, three type II and three type III cases. Haplotype analysis using microsatellite DNA markers flanking the SMA locus revealed that the 4bp deletion was found on the background of a same haplotype suggesting that a single mutational event was involved in the seven families. Extremely different phenotypes were found in two of these families: in one consanguineous familiy, one homozygous daughter was minimally affected while her three homozygous sisters were wheelchair bounding since their infancy. In the second family, while a 65 years old male showed a mild phenotype, his sister was wheelchair bounded during adolescence. To determine factors influencing the final phenotype we have performed analysis of the gene H4F5, the nearest gene located at the 5' end of the SMN gene. Analysis of PCR products of marker 212 showed that 92% of the Spanish type I chromosomes had 0 or 1 PCR product in comparison with 64% of type II chromosomes and 46% of type III chromosomes. These results support the hypothesis that H4F5 may be a modifier gene for SMA. Expression studies of this gene in human CNS are in progress. Supported by FIS 98-556 and Telemarato TV3.

Program Nr: 601 from the 1999 ASHG Annual Meeting

Analysis of chromosomal breakpoints in patients with Rieger Syndrome: effects on PITX2. *D.G. Trembath[1], E.V. Semina[1], B.U. Zabel[2], J.C. Carey[3], D.H. Jones[1], J.C. Murray[1]*. 1) University of Iowa, Iowa City, IA; 2) University of Mainz Hospital, Mainz, Germany; 3) University of Utah, Salt Lake City, UT.

Rieger syndrome is an autosomal dominant disorder characterized by eye abnormalities and dental hypoplasia. Semina et al. cloned the gene responsible for Rieger syndrome, *PITX2*, using two patients with balanced translocations near the gene: one between 4q26 and 16q22 and the other between 4q27 and 11q21. It is our hypothesis that these translocations either produce chromosomal rearrangements that delete *PITX2* or interrupt regulatory elements necessary for proper *PITX2* expression.

Using a cosmid map of 4q25-27, we subcloned the breakpoint regions on chromosome 4. We then cloned the 16:4 and 4:11 breakpoints via panhandle PCR (PH PCR), a novel technique for amplifying unknown regions of DNA. The 16:4 breakpoint is 18 kb telomeric to the 5'UTR of *PITX2* on chromosome 4 and occurs in a long terminal repeat (LTR). Using chromosome 16 sequence obtained from PH PCR, we identified a BAC covering the translocation region. The translocation on 16 maps near CHLC marker GATA71F09 and a novel CA repeat. To analyze the 4:16 translocation sequence, we have attempted to amplify this region from the patient's genomic DNA. This has revealed an apparent rearrangement of chromosome 16 sequence, resulting in a deletion of the 18 kb sequence telomeric of *PITX2* and possibly the gene itself.

The 4:11 breakpoint occurs in an Alu repetitive element approximately 60 kb telomeric of *PITX2* on chromosome 4, and in a MIR repetitive element on chromosome 11, between markers D11S901 and D11S1354. The 4:11 translocation creates a novel GATA binding site at the breakpoint that may potentially down regulate *PITX2*.

Future work will include delineating the nature of the potential deletion on the 4:16 chromosome and transient transfection studies to determine if down regulation of *PITX2* is occurring on the 4:11 chromosome.

Program Nr: 602 from the 1999 ASHG Annual Meeting

Functional haploinsufficiency of the elastin gene (ELN) in patients with isolated supravalvular aortic stenosis (SVAS). *Z. Urban[1], E.C. Davis[2], J. Zhang[3], M.R. Wallace[3], V.V. Michels[4], S.N. Thibodeau[4], B. Eyskens[5], K. Devriendt[5], C.D. Boyd[1]*. 1) Pacific Biomedical Res Ctr, Univ Hawaii, Honolulu, HI; 2) Cell Biology and Neuroscience, University of Texas Southwestern Medical Center, Dallas, TX; 3) Pediatric Genetics, University of Florida, Gainesville, FL; 4) Medical Genetics, Mayo Clinic, Rochester, MN; 5) Center for Human Genetics, Leuven, Belgium.

Supravalvular aortic stenosis (SVAS) is an autosomal dominant segmental stenotic arterial disorder caused by mutations in the elastin gene (ELN). We've previously characterized eight ELN mutations in families with isolated SVAS. Most of these mutations were point mutations resulting in premature termination codons. To study the molecular mechanism underlying SVAS, we've analyzed the expression of the elastin gene in skin fibroblast samples from SVAS patients. We've shown by RT-PCR analysis that a splice site mutation (IVS15-3CtoG), a frame shift mutation (1195delG) and a base substitution (1829GtoA, 610ArgtoGln), all result in a marked reduction or complete lack of expression of the mutant allele. The first two mutations resulted in at least 100-fold reduction in allele specific expression while mutation 1829GtoA resulted in a 4-8-fold reduction. These results were consistent with nonsense mediated decay (NMD) of the products of mutations IVS15-3CtoG and 1195delG and an as yet unknown mechanism affecting the stability of the mRNA products of the 1829A allele. Furthermore, we've demonstrated by Northern blot analysis that mutations result in an overall reduction of the steady state levels of elastin mRNA. Reduction of tropoelastin (the soluble precursor of elastin) synthesis and secretion paralleled the reduced steady state mRNA levels in SVAS fibroblasts assayed by metabolic labeling and immunoprecipitation. Finally, electron microscopic analysis of dermal elastic fibers in SVAS patients indicated an altered structure with discontinuities in the amorphous (elastin) component of the fiber. We conclude that despite molecular differences of the mutations responsible for SVAS, the unifying mechanism underlying this disorder is functional haploinsufficiency.

Program Nr: 603 from the 1999 ASHG Annual Meeting

Molecular and cellular defects in the CLN1 and CLN5 disorders. *J. Vesa[1], T. Klockars[2], J. Isosomppi[2], M. Savukoski[2], O. Heinonen[2], A. Kyttala[2], A. Jalanko[2], L. Peltonen[1].* 1) Human Genetics, UCLA School of Medicine, Los Angeles, CA; 2) Human Molecular Genetics, National Public Health Institute, Helsinki, Finland.

Neuronal ceroid lipofuscinoses (NCL) are a group of genetic neurodegenerative disorders caused by a defect in different genes, five of which have been identified by positional cloning. Clinically the most severe are two childhood forms, CLN1 and CLN5, both enriched in the Finnish population. A mutation in palmitoyl protein thioesterase (PPT) gene leads to CLN1 and a mutation in a novel gene is underlying CLN5. Over twenty mutations in the PPT gene have been reported, Arg122Trp being the most prevalent representing 98% of Finnish CLN1 alleles. PPT is an N-glycosylated lysosomal enzyme and its function is to remove palmitate groups from proteins. Wild-type PPT is targeted to lysosomes via mannose 6-phosphate receptor-mediated pathway and in vitro expression of PPT in mouse primary neurons shows transport of PPT to neuronal processes as well as co-localization with a presynaptic vesicle membrane protein, synaptophysin. A significant amount of PPT gets also secreted in both fibroblasts and primary neurons. Monitoring of mRNA levels by in situ hybridization of mouse brain sections revealed notable increase of PPT transcript postnatally just before the early synaptogenic period. The highest expression was found in the cerebral cortex, hippocampal cells, dentate gyrus granule cells and hypothalamus. The recently isolated CLN5 gene encodes a 46 kDa polypeptide with two potential transmembrane domains. Three disease mutations have been characterized: one 2 bp deletion enriched in Finland, one nonsense and one missense mutation. Based on the preliminary immunofluorescence data, wild-type CLN5 is intracellularly located in vesicles, most probably representing components of endocytotic pathways. Interestingly, three other CLN proteins represent lysosomal proteins: CLN1 and CLN2 are soluble enzymes and CLN3 is a membrane protein. Similar intracellular targeting of CLN proteins suggests their interactions or participation in the same metabolic pathways essential for normal neocortex development.

Program Nr: 604 from the 1999 ASHG Annual Meeting

A cellular assay distinguishes normal and mutant TIGR/myocilin protein. *D. Vollrath, Z. Zhou.* Dept of Genetics, Stanford Univ School of Medicine, Stanford, CA.

Glaucoma is a blinding eye disease that affects approximately 70 million people worldwide. Mutations in the gene *TIGR/MYOC* have been shown to cause the most common form of the disease, primary open angle glaucoma, in selected families. Amino acid sequence variants of the gene have been found in 2-4% of sporadic primary open angle glaucoma cases. However, of the over 40 amino acid variants described, most are rare and it is often difficult to definitively distinguish between a deleterious mutation and a benign variant solely on the basis of relative frequencies in patient and control groups. The function of the TIGR/myocilin protein is unknown and an assay to functionally classify variants is lacking. We sought to develop a biochemical assay to distinguish different forms of TIGR/myocilin. We investigated the Triton X-100 detergent solubility characteristics of mutant and normal forms of the protein, expressed by transfection in cell culture. We observed a clear difference in the behavior of the two types of TIGR/myocilin; all 12 mutant proteins tested were substantially Triton insoluble, while normal protein and controls were completely soluble. We also tested seven ambiguous variant proteins and classified them as mutant or normal on the basis of their Triton solubility. The results in some cases validated, and in other cases contradicted, earlier classifications of these variants. To our knowledge, Triton solubility is the first example of a general difference in the properties of mutant and normal forms of TIGR/myocilin. The assay we have developed will be useful for discerning protein functional information from the location of mutations, will aid genetic counseling of individuals with TIGR/myocilin variants, and may provide a clue to understanding the mechanism by which mutations in *TIGR/MYOC* cause glaucoma.

Program Nr: 605 from the 1999 ASHG Annual Meeting

Analysis of Spinocereballar Ataxia Type 1 (SCA1) Knock-in mice. *K. Watase[1,2], D. Lorenzetti[2], B. Xu[2], D. Armstrong[2], H.T. Orr[3], H.Y. Zoghbi[1,2].* 1) Howard Hughes Medical Institute; 2) Baylor College of Medicine, Houston, TX; 3) University of Minnesota, Minneapolis, MN.

Spinocerebellar ataxia type 1 (SCA1) is an autosomal dominant neurodegenerative disorder characterized by progressive ataxia, and selective neuronal loss within the cerebellar cortex and brain stem. To elucidate the pathophysiology of SCA1 and to evaluate repeat length instability in the context of the SCA1 genomic locus, we generated knock-in mice by inserting an extended tract of 78 CAG repeats into the mouse *Sca1* locus ($Sca1^{78Q}$). The levels of mutant ataxin-1 in $Sca1^{78Q/2Q}$ mice are comparable to the levels of the endogenous protein. Histological examination of brain tissue from mutant mice did not reveal detectable neuropathological changes; immunohistochemical analysis with anti ataxin-1 antibodies failed to detect neuronal intranuclear inclusions in mutants up to 15 months of age. Neither $Sca1^{78Q/2Q}$ nor $Sca1^{78Q/78Q}$ mice show any overt ataxic phenotype. Analysis of motor performance on the rotating rod was done on $Sca1^{78Q/78Q}$ mice (n = 7) and wild type (WT) littermates (n = 5) at 3, 6, and 9 months of age. Both groups performed similarly at 3 and 6 months, however at 9 months $Sca1^{78Q/78Q}$ mice had reduced mean time on the rotating rod compared to WT (112 ± 16 sec vs 261 ± 37 sec, respectively, $p < 0.05$). $Sca1^{78Q/2Q}$ mice show intergenerational repeat instability (+2 to -6) at a frequency of 10% in paternal transmissions (n = 90), and 65% in maternal transmissions (n = 62). Contraction accounted for 39/40 changes and the intergenerational instability increased in frequency and magnitude as the mother aged. In paternal transmissions, expansions and contractions were observed at similar frequencies. These data suggest that the toxicity of mutant ataxin-1 when expressed at endogenous levels is only manifested after prolonged exposure which is reminiscent of the human disease (78Q causes disease ^{3} 4 years). Although large intergenerational expansions or contractions were not observed in the $Sca1^{78Q/2Q}$ mice, the pattern and direction of repeat instability are similar to those observed in human SCA1.

Program Nr: 606 from the 1999 ASHG Annual Meeting

Glypican 3 (GCP3) deletions: the phenotypic spectrum in overgrowth syndromes. *R. Weksberg[1], M. Li[1], C. Shuman[1], E. Cutiongco[1], H.A. Bender[2], C. Stevens[3], L. Wilkins-Haug[4], S.L. Wong[5], J. Squire[1]*. 1) Division of Clinical & Metabolic Genetics, Hospital for Sick Children, University of Toronto, Toronto, ON, Canada; 2) Regional Genetics Center, South Bend, IN; 3) T.C. Thompson Children's Hospital, Chattanooga, TN; 4) Brigham & Women's Hospital, Boston, MA; 5) Dept. of Medical Genetics, University of British Columbia, Vancouver, BC, Canada.

Simpson-Golabi-Behmel syndrome (SGBS) is an X-linked overgrowth syndrome caused by deletions in GPC3, a cell surface proteoglycan believed to function in modulating growth factor activity. SGBS is characterized by pre-and postnatal overgrowth, a characteristic facies and a spectrum of congenital malformations which overlaps other overgrowth syndromes. Using clinical criteria, we selected 79 male overgrowth patients in the following categories: SGBS (n=18), query SGBS (n=26) and Beckwith-Wiedemann syndrome (BWS) (n=35). Using exon-specific PCR and Southern blot analysis, we identified 5 GPC3 deletions among the 18 SGBS patients. In none of these cases was a clear family history present and 1 patient had an atypical facies for SGBS. In the screen of 26 query SGBS patients, 1 GPC3 deletion was identified in a patient belonging to a pedigree in which a maternal first cousin had previously been diagnosed with Perlman syndrome. No GPC3 deletions were identifed in a screen of 35 BWS patients. Among our results and all the GPC3 deletions published to date, there are no clear phenotypic correlations for specific deleted exons. However, our results validate the specificity of the following clinical findings in patients with GPC3 deletions: skeletal and hand anomalies and supernumerary nipples. Our data also indicates that nephroblastomatosis should be included in the phenotypic spectrum of GPC3 deletions and SGBS. Given that the combined frequency of GPC3 deletions in our patients and the literature is only 33%, we directly sequenced all GPC3 exons in 10 SGBS patients and failed to identify any mutations, raising the possibility of alternative silencing mechanisms and/or other genes in the pathogenesis of SGBS.

Program Nr: 607 from the 1999 ASHG Annual Meeting

X-Linked Hypophosphatemia (XLH): Mutations Compromising PHEX Structure Reflect A Severe Phenotype.

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XLH is the most common heritable form of rickets. Approximately 100 different mutations compromising the structure of the PHEX (phosphate-regulating gene with homologies to endopeptidases on the X-chromosome) gene product have been reported in patients worldwide. Nevertheless, PHEX gene defects have not been identified in approximately 33 percent of patients and the pathogenesis of this sex-linked dominant disorder is unclear because the substrate for the putative PHEX-encoded endopeptidase has not been characterized. To explore variable disease severity among XLH families, we performed phenotype/ genotype analysis using a large population of children treated at one institution during the past 15 years. All were receiving therapy with 1,25(OH)2D3 and phosphate supplementation. To date, 60 unrelated probands have had molecular studies of the 2,247 bp PHEX coding region. Different gene defects have been identified in 25 of these XLH families. However, in 13 additional XLH families RT-PCR with DNA sequence analysis failed to reveal a PHEX mutation, thereby excluding a defect in the coding region of the PHEX gene. XLH probands with PHEX mutations were compared to XLH probands in whom no PHEX mutation was identified. Height and arm span Z-scores were used as the principal clinical determinants of XLH severity. TmP/GFR was used as an independent (biochemical) determinant. Medically-treated patients with vs without PHEX mutations had different height and arm span Z-scores (-1.80 vs -0.95, $p = 0.036$ and -1.67 vs -0.61, $p = 0.019$, respectively) and TmP/GFR values (1.90 vs. 2.30, $p = 0.016$, respectively). At diagnosis (untreated) height Z-scores were different (-1.80 vs. -0.41, $p = 0.011$). Only children with mutations had undergone osteotomies. We conclude that disruption of the PHEX gene product is associated with the most severe phenotype in XLH.

Program Nr: 608 from the 1999 ASHG Annual Meeting

The spinal muscular atrophy protein, SMN, fails to form nuclear gems in some mammalian cell types which also lack coiled bodies. *P.J. Young[1], T.T. Le[2], N.T. Man[1], A.H.M. Burghes[2], G.E. Morris*[1]*. 1) MRIC Biochemistry Group, North East Wales Institute, WREXHAM, UK; 2) Dept of Medical Biochemistry, Ohio State University, COLUMBUS, OH.

Spinal muscular atrophy (SMA) is caused by mutations in a gene encoding SMN (survival of motor neurons) protein, leading to reduced SMN levels and loss of motor neurons. SMN is present in the cytoplasm of all cells and in distinct nuclear structures called gems, which associate with nuclear coiled bodies (CBs) and have an indirect role in pre-mRNA splicing. The distribution of nuclear gems and CBs was determined in human, rabbit and pig tissues using monoclonal and polyclonal antibodies against SMN, SMN-interacting protein-1 (SIP-1) and coilin p80 protein. Although SMN and SIP-1 are present in all tissues, nuclear gems were not detected in cardiac and smooth muscles, blood vessels, stomach, skin and spleen. Gems were most abundant in larger neurons of the brain and spinal cord, but they were also present in skeletal muscle, glial cells, liver parenchymal cells, islets of Langerhans and several epithelial cell types. Nuclear CBs were only found in those cell types which also contain gems. Over-expression of SMN in COS-7 cells produced supernumery nuclear bodies which also contained SIP-1 and coilin p80, confirming the close relationship between gems and CBs. However, gems are not essential for CB formation since fibroblasts from SMA patients still contained CBs, although SMN was undetectable and SIP-1 remained in the cytoplasm.
We conclude that gem formation is not determined by high cytoplasmic SMN concentrations alone and a cell-specific factor may be required for formation of both gems and CBs. Our data support the view that nuclear bodies are storage sites which are not essential for nuclear RNA splicing. *We thank the Muscular Dystrophy Association (USA) for grant support, Angus Lamond (University of Dundee) for p80 antibodies and Matt Dunckley (Imperial College School of Medicine, London) for human and pig tissues. .*

Program Nr: 609 from the 1999 ASHG Annual Meeting

The ABCR gene in recessive and dominant Stargardt diseases: a genetic pathway in macular degeneration. *K. Zhang[1], D.C. Garibaldi[1], M. Kniazeva[2], A. Hutchinson[2], M. Han[3], M. Dean[3], R. Allikmets[3,4]*. 1) Wilmer Eye Inst, Johns Hopkins Medical Inst, Baltimore, MD; 2) Dept of Molecular, Cellular and Developmental Biology, Univ of Colorado, Boulder, CO; 3) Laboratory of Genomic Diversity, National Cancer-Institute-FCRDC, Frederick, MD; 4) Dept of Ophthalmology and Pathology, Columbia Univ., New York, NY.

Stargardt disease (STGD) is a juvenile onset macular dystrophy which can be inherited in both an autosomal recessive and dominant manner. Genes involved in dominant STGD have been mapped to human chromosomes 13q (STGD2), 6q (STGD3) and 4p (STGD4). Here, we identify a new kindred with dominant STGD, and demonstrate genetic linkage to the STGD3 locus. Because of a more severe phenotype of one of the patients in the family, the gene responsible for recessive STGD1, ABCR, was analyzed for sequence variants in all family members. One allele of the ABCR gene was shown to carry a stop codon-generating mutation (R152X) in three family members, including the patient who had also inherited STGD3. More specifically, the combination of alleles from both the STGD1 and STGD3 loci appear to result in earlier onset of disease, with a more severe clinical phenotype. A grandparent of the patient with both the STGD1 and STGD3 mutations developed age-related macular degeneration (AMD), consistent with our earlier observation that some variants in the ABCR gene may increase susceptibility to AMD in the heterozygous state. Based on these results, we propose that there is a common genetic pathway in macular degeneration that includes genes for both recessive and dominant STGD.

Program Nr: 610 from the 1999 ASHG Annual Meeting

Variable loss of function of OSF2/CBFA1 correlates with variable expressivity in cleidocranial dysplasia and primary dental abnormalities. *G. Zhou[1], Y. Chen[1], L. Zhou[1], C. R. Greenberg[2], K. Thirunavukkarasu[1], G. Karsenty[1], B. Lee[1]*. 1) Dept. of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Dept. of Human Genetics, University of Manitoba, Winnipeg, MB.

Cleidocranial dysplasia (CCD) is a dominantly inherited skeletal dysplasia characterized by hypoplastic or absent clavicles, large fontanelles, dental anomalies, and delayed skeletal development. Previously we and other groups reported that mutations in the osteoblast-specific transcription factor OSF2/CBFA1 cause CCD. To further correlate OSF2/CBFA1 mutations in different functional domains with the CCD phenotype, we studied twenty-six independent cases of CCD and a total of fifteen new mutations were identified. The majority are missense mutations that affect conserved residues in the runt domain and abolish DNA binding by electrophoretic mobility shift assay (EMSA). These and mutations which result in premature termination in the runt domain produce the phenotype by haploinsufficiency of OSF2/CBFA1. Additionally, we have identified a R391X mutation which encodes for a protein truncated for half of the putative transactivation PST domain. Interestingly, we have found a putative hypomorphic mutation (a T200A substitution) in a highly conserved position of the runt domain which does not affect DNA binding or transactivation in vitro. In this family the father only has primary teeth anomalies, while the sons have the classical CCD skeletal features. This may represent a third type of mutation producing a hypomorphic effect associated with significant clinical variability including an isolated dental phenotype. Alternatively, this might signify alteration of an independent function such as heterodimerization which has been previously attributed to the runt domain. Together these data show that loss of function due to alteration in all three domains (Runt, PST, Q/A) of OSF2/CBFA1 gives rise to clinical variability in the CCD phenotype, and in some cases, mutations may be associated with primary dental anomalies.

Program Nr: 611 from the 1999 ASHG Annual Meeting

Advantages of FISH for characterising delivery of helper-dependent adenoviral vectors for gene therapy. *O.A. Bodamer, B. Mull, B. Lee, A.L. Beaudet.* Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX.

Fluorescence in Situ Hybridization (FISH) is a widely used molecular technique for the detection of human DNA sequences and their localization on metaphase chromosomes and interphase nuclei. Probe preparation typically relies on high quality human DNA from YAC, PAC, BAC or cosmid contigs. We report on the use of FISH for the detection of viral DNA within interphase nuclei, an approach which may allow assessment of efficacy of gene transfer. Human fibroblasts were infected with increasing concentrations of helper-dependent adenoviral vector containing the BOS-ASS (argininosuccinate synthetase) cDNA and human genomic stuffer DNA (100 MOI, 1000 MOI, 10000 MOI) for 4 hrs, 12 hrs, 24 hrs, 48 hrs and 96 hrs respectively. Helper virus contamination of BOS-ASS vector preparation was minimal (~5%) as judged by Southern blotting. FISH was done using the standard FISH protocol currently in use at the Cytogenetics Laboratory at Baylor College of Medicine. BOS-ASS vector and helper virus particles were detected following the in situ hybridization of extracted interphase nuclei by the use of digitoxin labeled adenoviral backbone and helper virus DNA respectively. There was good correlation between the dose of infection and the number of FISH signals for BOS-ASS vector and helper virus respectively. Helper-dependent adenoviral vectors are hard to titer because they do not form plaques. FISH allows a biologically meaningful direct measurement of the number of vector and helper genomes delivered to tissues such as liver and on metaphase cells to determine if vector DNA is integrated in the host genome or not. Both viral DNA sequences can also be conveniently detected using two-color FISH. FISH may consequently play an important role for the *in-vivo* assessment of gene transfer in human trials.

Program Nr: 612 from the 1999 ASHG Annual Meeting

Synergistic effect of histone hyperacetylation and DNA demethylation in the reactivation of the FMR1 gene. *P. Chiurazzi[1], M.G. Pomponi[1], R. Pietrobono[1], C.E. Bakker[2], G. Neri[1], B.A. Oostra[2].* 1) Institute of Medical Genetics, Catholic University, and Centro Ricerche per la Disabilita' Mentale e Motoria, Associazione Anni Verdi, Rome, Italy; 2) Department of Clinical Genetics, Erasmus University, Rotterdam, The Netherlands.

Most fragile X syndrome patients have an expansion of a (CGG)n sequence with more than 200 repeats (full mutation) in the promoter region of the FMR1 gene, responsible for this condition. Hypermethylation of the expanded repeat and of the FMR1 CpG island is almost always present and apparently suppresses transcription, resulting in the absence of the FMR1 protein. We recently showed that transcriptional reactivation of FMR1 full mutations can be achieved by inducing DNA demethylation with 5-azadeoxycytidine (5-azadC). Given that the level of histone acetylation is another important factor in regulating gene expression, we treated lymphoblastoid cell lines from nonmosaic full mutation patients with three drugs capable of inducing histone hyperacetylation. We report a consistent, although quantitatively modest, reactivation of FMR1 with 4-phenylbutyrate (4-PBA), sodium butyrate (BA) and trichostatin A (TSA), as shown by RT-PCR. However, combining these drugs with 5-azadC results in a 2- to 5-fold increase of FMR1 mRNA levels obtained with 5-azadC alone, thus showing a marked synergistic effect of histone hyperacetylation and DNA demethylation. Lastly, we treated the cell lines with acetyl-L-carnitine (ALC), a compound with a chemical structure similar to that of butyrate. The effect of ALC on FMR1 reactivation was similar to that of 4-PBA and BA. It is worth noting that ALC, an approved drug, was found to be effective in the treatment of hyperactivity in an experimental animal model. Preliminary data suggest that a similar effect is present in fragile X syndrome patients treated with ALC. P.C. is recipient of Telethon fellowship 283B. Supported by Telethon grant E.245 to G.N. and Associazione Anni Verdi, Roma.

Program Nr: 613 from the 1999 ASHG Annual Meeting

Mammalian artificial chromosome ST1. *M.H. Shen[1], J. Mee[2], F. Brook[3], J. Nichols[2], J. Yang[1], A. Smith[2], R. Gardner[3], W. Brown[1]*. 1) Dept Biochemistry, Univ Oxford, Oxford OX1 3QU, England; 2) Centre for Genome Research, Univ Edinburgh, Edinburgh EH9 3JQ, Scotland; 3) Dept Zoology, Univ Oxford, Oxford OX1 3PS, England.

Generation of a mammalian artificial chromosome (MAC) should help us understand the DNA sequence elements required for chromosome function during mitosis and meiosis. MACs may also be useful as vectors for introducing large DNA fragments into cells or animals. We have engineered a 4.5 Mb mini-chromosome, termed ST1, that consists of 3.0 Mb mouse centromeric DNA and 1.5 Mb human DNA including an array of <100 kb alphoid satellite sequence. ST1 is structurally and mitotically stable in cultured cells of chicken, murine or human origin. We have transferred ST1 into mouse ES cells and established chimeric animals from these cells. The mini-chromosome is stable in these animals and can be transmitted through the female germ line. These results help define the molecular and cellular requirements for MAC construction.

Program Nr: 614 from the 1999 ASHG Annual Meeting

Neural stem cells for the treatment of the twitcher mouse model of Krabbe disease. *D.A. Wenger[1], E.Y. Snyder[2], R.M. Taylor[3], M.T. Vanier[4], M.A. Rafi[1], P. Luzi[1], J. Datto[1]*. 1) Dept Neurology, Jefferson Medical Col, Philadelphia, PA; 2) Dept Neurology, Harvard Medical School, Boston, MA; 3) Faculty of Veterinary Sci, University of Sydney, Australia; 4) INSERM U189, Oullins, France.

Krabbe disease is an autosomal recessive disorder of humans and certain animals, including twitcher (twi) mice, caused by the deficiency of galactocerebrosidase (GALC) activity. This lysosomal enzyme is responsible for the degradation of certain galactolipids, gal-ceramide and psychosine. The accumulation of psychosine in white matter causes death to oligodendrocytes (OLG) resulting in the loss of myelin and presence of characteristic globoid cells. Therefore therapy strategies could include either supplying GALC activity to OLG healthy enough to produce myelin, or replacement of dead or dying OLG with new ones that contain GALC activity. Neural stem cells (NSC) are immature brain cells that can be cultured, and once injected into the brain, can differentiate into several cell types, including OLG. Initially, unmodified NSC, (clone C17.2), were injected intracerebroventricularly into newborn twi mice. The cells engrafted throughout the brain and differentiated into healthy OLG. Donor cells can be detected by a X-gal histochemical reaction. In regions of NSC engraftment, normal appearing myelin can be clearly visualized, while axons in non-engrafted areas do not show myelination. Because these mice did not show complete phenotypic or biochemical correction, NSC were transduced with a retroviral vector containing human GALC cDNA (MFG-GALC). Three subpopulations of NSC were isolated, and these express 78, 94 and 99 nmol/h/mg prot of GALC activity vs 5-7 in untransduced NSC clones. These will have more GALC activity to donate to OLG of the recipient. At this time all newborn mice from 7 litters of heterozygous twi matings have been injected with over-expressing NSC. Twi mice from currently injected litters will be evaluated clinically, and the brains will be examined biochemically for GALC activity and lipids, and neuropathologically for myelination and the presence of GALC antigen. The successful use of NSC for this disorder has implications for the treatment of other neurodegenerative disorders.

Program Nr: 615 from the 1999 ASHG Annual Meeting

Fine mapping of a neuroblastoma tumor suppressor candidate gene region in 1p36.2-3, and mutation analysis of the cortistatin gene localized to the region. *F. Abel[1], K. Ejeskar[1], R.M. Sjoberg[1], P. Kogner[2], T. Martinsson[1]*. 1) Dept Clin Genetics, Sahlgrenska Univ Hosp, Gothenburg, Sweden; 2) Childhood Cancer Research Unit, Karolinska Hosp, Stockholm, Sweden.

Neuroblastoma is a childhood tumor originating from the neural crest. A common genetic feature of neuroblastomas, also being an important prognostic factor, are deletions of chromosome region 1p. The deletion of 1p often involves a deletion of varying size, with a consensus region within the most distal bands 1p36.2-3. The neuroblastoma SRO (shortest region of overlap of deletions) presented earlier by our group is defined distally by the cluster of loci D1S80/D1Z2/CDC2L1 and proximally by loci D1S244 i.e. app. 25 cM. The 1p deletions are, however, not restricted to neuroblastoma tumors. In fact a large spectrum of tumor types display deletions to varying degree of distal 1p. We have exploited the possibility of using deletions of other tumor types, preferentially that of germ cell tumors, and combining those with that of the neuroblastoma SRO. Also in germ cell tumors distal 1p-deletion have been shown to have prognostic significance. We found in our germ cell tumors a SRO ranging from D1S508 to D1S200. Interestingly, this region only partially overlapped (app. 5cM) with our neuroblastoma SRO in region D1S508 to D1S244. We have thus focused on analyzing this smaller region in search for genes involved in the genesis of different cancers. We have performed radiation hybrid mapping of a large number of markers, STSs, ESTs and others known to reside in 1p. We have also initiated the development of a BAC contig of the region. FISH, and fiber-FISH mapping of BACs were also performed. Cortistatin (CORT) is highly homologous to somatostatin, and the presence of somatostatin in neuroblastoma tumors has been shown to have a favorable prognostic value. Here we have localized CORT to the neuroblastoma consensus region, defined the genomic organization of the gene and performed mutation analyses and gene expression studies of neuroblastoma primary tumors. No evidence has yet however been found to confirm CORT to be a neuroblastoma tumor suppressor gene.

Program Nr: 616 from the 1999 ASHG Annual Meeting

Identification of Thy-1 as a putative tumor suppressor gene for ovarian cancer. *H.R. Abeysinghe[1], S.J. Pollock[1], P. Keng[2], J. Xu[1], M. Halterman[3], H.J. Federoff[3], N.L. Guckert[1], B. Cohen[1], N. Wang[1]*. 1) Dept. of Pathology and Laboratory Medicine; 2) Dept. of Radiation Oncology; 3) Center for Aging and Development Biology, University of Rochester, School of Medicine and Dentistry, Rochester, NY 14642.

Microcell-mediated transfer of chromosome 11 into the human ovarian cancer cell line SKOV-3 results in complete and partial suppression of tumorigenicity in mice. To identify the differentially expressed transcripts associated with tumor suppression, cDNA population from the slow-growing tumorigenic clone 11(H)8-3, tumorigenic clone 11(H)8-4 and SKOV-3 cells were subtracted from the non-tumorigenic clones, 11(H)7-2 and 11(C)9-8. The differentially expressed transcripts were cloned, sequenced, characterized, and their expression confirmed by northern analysis. A cDNA transcript corresponding to the Thy-1 gene, located at chromosome 11q23-24, was found to be exclusively expressed in the two non-tumorigenic clones, 11(H)7-2 and 11(C)9-8. This expression pattern was confirmed by immunocytochemistry and flow cytometry at the protein level. Thy-1 was also shown to be expressed in normal ovarian tissue. However, no Thy-1 expression was detected in five ovarian tumor lines examined. The entire cDNA of the Thy-1 gene was cloned and was found to be different from the published sequence. The cloned Thy-1 sequence was confirmed in DNA samples derived from both normal male and female. Transfection of Thy-1 cDNA into SKOV-3 with its subsequent expression reduced tumor growth but did not suppress it completely. However, transfection of Thy-1 antisense into cells from the non-tumorigenic clone 11(C)9-8 resulted in almost complete inhibition of expression, and the restoration of tumorigenicity in two of three animals inoculated. Cells derived from these two tumors did not express any detectable Thy-1. These observations indicate that Thy-1 is a putative tumor suppressor gene for ovarian cancer.

Program Nr: 617 from the 1999 ASHG Annual Meeting

Retrospective analysis of the Wijnen et al. logistic model in 43 HNPCC families. *K. Aittomaki[1], C. Gaff[1], J. St.John[2], F. Macrae[2], R.J.M. Gardner[1].* 1) Victorian Clin. Genet. Service, Melbourne, Victoria, Australia; 2) Department of Gastroenterology, Royal Melbourne Hospital, Australia.

Genetic testing for HNPCC remains complicated with a large number of mutations scattered along several genes. Special features of family history, MSI testing and immunohistochemistry can be used as tools to select families for genetic testing. Based on data from 184 families with 47 mutations in MLH1 and MSH2 Wijnen et al have developed a logistic model to calculate the probability of finding a mutation in a given family using the mean age at diagnosis of colorectal cancer (CRC), Amsterdam positive/negative and presence/absence of endometrial cancer as criteria. They suggest that direct genetic testing without prior MSI should be done in families with ³ 0.2 likelihood of finding a mutation. In others, MSI testing should be performed prior to genetic screening. To evaluate the usefulness of the Wijnen et al. logistic model, we have retrospectively analysed 43 HNPCC families with the model. Of these, 20 families have an identified mutation whereas 23 had a negative PTT result for mutations in MLH1 and MSH2. The mean age at diagnosis of CRC was 44.1 years in families with a mutation and 53.7 in mutation negative families. In both groups 12 families were positive for Amsterdam criteria and the number of families with endometrial cancer was 8 and 5, respectively. When analyzed, 75% (15) of the mutation positive families had a probability score of 0.2 or higher. In 74% (17) of the mutation negative families the score was lower than 0.2 suggesting the use of MSI testing prior to screening for mutations. We conclude that the Wijnen et al. logistic model can be used as a screening method for direct genetic testing for HNPCC. However, it is important to realize that it cannot be used as a tool for selecting families for genetic testing. The purpose of this model is to help in minimizing the need for other tests such as MSI. This is particularly important in countries like Australia where due to migration families have relatives overseas and accessing tumour blocks for MSI testing is often difficult, time consuming and expensive, if not impossible.

Program Nr: 618 from the 1999 ASHG Annual Meeting

False negative results in BRCA testing from paraffin-embedded tumors. *M. Alvarez-Franco, E. Matloff, A.E. Bale.* Genetics, Yale University, New Haven, CT.

BRCA 1 & 2 testing is often sought by healthy women in high-risk families who wish to determine if they are at increased risk for breast/ovarian cancer. Three mutations (185delAG & 5382insC in BRCA1; 6174delT in BRCA2) are found frequently in Ashkenazi Jews. Typically an affected family member, who can serve as a positive control, is tested along with at-risk relatives. Testing unaffected individuals without testing affected relatives can lead to ambiguous results. A negative test may indicate that the subject is in fact a true negative, the family carries a mutation not detected by the test, or breast cancer in the family represents a sporadic clustering. In kindreds with no living affected members, archived pathology specimens can be analyzed as a means of determining whether one of these mutations segregates in the family. We obtained tissue blocks from deceased probands in 11 Ashkenazi Jewish families in which no living, affected relatives were available for testing. DNA extracts from blocks were analyzed for the mutations by PCR and direct sequencing of BRCA1 exons 2 and 20 and BRCA2 exon 11. 185delAG was found in 3 samples, 5382insC in 2, and no mutation in 4. Two blocks failed to amplify, even with nested primers. Heterogeneity of tumor tissue was a potential problem in this study. In one of the families, a 5382insC mutation was detected in one region of a breast tumor while another area was negative. It is very unlikely that this result represents a somatic, tumor-specific genetic alteration resulting in creation of a founder mutation. Although unusual, it appears that portions of this tumor lost the mutant 5382insC allele. To evaluate the prevalence of this phenomenon, tissue blocks from other families with known mutations were tested. In a family with a 185delAG mutation, paraffin-embedded colon tumor tissue lost the mutant allele almost completely. Testing tissue blocks for founder mutations in Ashkenazi Jews and other ethnic groups should be considered in families with no living, affected relatives. If tumor tissue is used as a source of DNA, then several regions of the tissue should be sampled for the presence of mutations.

Program Nr: 619 from the 1999 ASHG Annual Meeting

Identification of differentially expressed genes which are down regulated in human malignant mesothelioma (MM) using suppression subtractive hybridization (SSH). *S. Apostolou, J. Klein, D. Roberts, J.R. Testa.* Medical Oncology, Fox Chase Cancer Center, Philadelphia, PA.

MM is a highly aggressive mesodermal neoplasm characterized by a long latency following exposure to asbestos. The length of the latent period suggests that multiple genetic alterations may be required for tumorigenic conversion of a mesothelial cell. The most frequent sites of chromosomal loss in MM include 1p21-p22, 3p21, 6q15-21 and 22q, implying that tumor suppressor genes (TSGs) reside in these regions.

To determine which TSGs are localized in these and other chromosome sites of interest, a PCR-based technique, SSH, was used to identify genes expressed in normal mesothelial cells (tester), but absent in MM (driver) cell lines. Following cDNA synthesis from tester and driver, cDNA was digested with *Dpn*II, and tester cDNA was separated into two independent adapter ligated populations. Each tester was hybridized with a different *Dpn*II digested driver, prior to being combined and incubated with an additional adapter-ligated tester/driver hybridization reaction. Tester:tester hybridization products, each containing single stranded cDNA from the individual adapter-ligated cDNA populations, were PCR-amplified and radiolabeled. The final difference products, representing the mRNA present in mesothelial cells but missing in MM cell lines, were separated by electrophoresis in denaturing polyacrylamide gels. Following autoradiography, 50 bands were excised from the gel, and the DNA was eluted then cloned. The inserts were PCR-amplified and sequenced to determine their identity. To validate this technique, 3 clones, annexin VIII, tissue plasminogen activator, and integrin, were randomly chosen to analyze their expression in MM compared to normal mesothelial cells by RT-PCR. These clones possessed differential patterns of expression in MM, including the MM cell lines used in the SSH experiment. Currently, the remaining 47 clones are being investigated for their role in MM. Undoubtedly, the use of SSH to identify differentially expressed genes will lead to a greater understanding of the molecular alterations in MM.

Program Nr: 620 from the 1999 ASHG Annual Meeting

The 8p22 tumor suppressor gene site: identification of novel expressed sequences. *Z.H. Arbieva[1], S.L. Edassery[1], K. Banerjee[1], S.Y. Kim[1], S.K. Horrigan[2], C.A. Westbrook[1]*. 1) Section of Hematology/Oncology, Department of Medicine, Univ Illinois at Chicago, Chicago, IL; 2) Department of Pediatrics, Lombardi Cancer Center, Georgetown University Medical Center, Washington, DC.

The short arm of chromosome 8 is a frequent site of allelic deletions in a wide variety of epithelial tumors. Clinical correlations, as well as functional chromosome transfer studies, have provided supporting evidence that this region contains a tumor suppressor gene (TSG) which is associated with invasiveness and/or tumorigenicity in prostate, colorectal, breast, and other types of cancers. An interval of approximately 1 Mb centered at the MSR marker on 8p22 has emerged as a likely site for TSG activity, based on the high degree of allelic loss and the presence of homozygous deletions in a metastatic prostate tumor sample and a pancreatic cancer cell line. To map this interval and identify candidate genes, we prepared a sequence-ready contig of bacterial clones, making an effort to integrate markers with the Radiation Hybrid Database (Rhdb). Transcribed units within the interval were identified by several strategies including placement of unmapped ESTs from Rhdb, exon amplification, as well as searching and mapping of ESTs from the Human Transcript Map and other published sources. Our effort resulted in the identification of seven unique expressed sequences situated within the target interval; six of them are expressed in a panel of epithelial cell lines and therefore represent viable candidates for the TSG. Analysis of available sequence to date has revealed that two ESTs show minor homology to known genes including a phosphokinase; the others have no known homologies. Full-length sequence assembly is ongoing, and has been completed for three clones. Mutational analysis of these genes and more detailed expression studies are now underway to test them as candidates for the 8p22 TSG.

Program Nr: 621 from the 1999 ASHG Annual Meeting

Deletion mapping identifies a major target site of LOH on chromosome 11q23 in cervical carcinoma. *H. Arias[1,2], J.M. Fakruddin[2], N. Beleno[1], G. Martinez[1], V.V.V.S. Murty[2].* 1) Dep. Immunology, Instituto Nacional de Cancerologia,Cl 1 No. 9-85. Bogota, DC, Colombia; 2) Department of Pathology, College of Physicians & Surgeons of Columbia University, 630 West 168th Street, New York, NY 10032.

Functional studies have shown that the chromosome 11 suppresses tumorigenicity in HeLa cells and other histologic types of cervical carcinoma where the critical region mapped to 11q13-q23. Loss of heterozygosity (LOH) studies has further suggested that one or more tumor suppressor genes relevant to cervical carcinoma maps to chromosome 11. In order to characterize allelic deletions and identify sites of candidate tumor suppressor gene(s), we performed a detailed deletion mapping of chromosome 11 using 30 normal-tumor DNAs from cervical carcinoma assaying 22 polymorphic markers mapped to both the short and long arms. Twenty-four (80%) tumors exhibited LOH at one or more loci with 40% at 11p and 70% at 11q had allelic deletions. The pattern of LOH identified 5 common sites of deletions, 2 on short arm (11p15 with 31%, 11p12-13 with 29%) and 3 on the long arm (11q13 with 37%, 11q23 with 60%, 11q25 with 44%). The region at 11q23 had highest frequency of LOH with the minimal region of deletion spanned by markers D11S4167, D11S1353, D11S4144 and D11S4094, a genetic distance of 6-cM. These data suggest that a candidate tumor suppressor gene lie within this interval that may be important in cervical carcinogenesis.

Program Nr: 622 from the 1999 ASHG Annual Meeting

Molecular characterization of FRAXB and comparative fragile site instability in tumor cell lines. *M.F. Arlt[1], J.M. Fang[1], D.G. Beer[2], T.W. Glover[1]*. 1) Departments of Pediatrics and Human Genetics; 2) Department of Surgery, University of Michigan, Ann Arbor, MI.

Common fragile sites are loci that show gaps or breaks on metaphase chromosomes when cells are grown under conditions that impair DNA synthesis. These fragile site regions span hundreds of Kb. The colocalization of common fragile sites and translocation breakpoints suggests that fragile sites play a role in genomic rearrangements seen in cancer. The best-studied common fragile site is FRA3B, lying within the *FHIT* gene at 3p14.2. *FHIT*/FRA3B shows many aberrations in cancer, including deletions, translocations, and aberrant splicing, suggesting that *FHIT* is a tumor suppressor gene. These studies have raised the question of what properties other common fragile sites and associated genes display in tumorigenesis. FRAXB is a frequently-expressed fragile site mapping to Xp22.3. We have identified YACs that span FRAXB. Like FRA3B, it spans hundreds of Kb. The STS gene is also contained on this YAC. We have investigated three common fragile sites (FRA3B, FRAXB, FRA7H) for deletions in tumor cell lines. Cell lines with *FHIT*/FRA3B alterations were chosen in order to evaluate the relative frequencies of instability at other fragile sites. About 33% of the cell lines had homozygous deletion of FRA3B sequences using genomic PCR assays. Hemizygous deletions at FRAXB were seen in 25% of cell lines. To date, no deletions have been detected at FRA7H. Southern blot analysis to detect hemizygous deletions is underway. We have also examined the expression of genes associated with fragile sites (FHIT at FRA3B; *STS*, *TLR5a*, and *GS1* at FRAXB) in these cell lines. Approximately 86% of cell lines had alterations or deletions in the *FHIT* transcript. *STS* and *GS1* were deleted in only 29% of cell lines and no aberrant transcripts were noted. *TLR5a* was not altered in the cell lines examined. We have shown that deletions occur at FRAXB in addition to FRA3B, suggesting that instability at fragile sites might be a general phenomenon in some tumors. In contrast, gene alterations are much more common at *FHIT*/FRA3B than at *STS*/*TLR5a*/FRAXB, possibly due to the position of FRA3B within the large *FHIT* locus.

Program Nr: 623 from the 1999 ASHG Annual Meeting

Mapping of Chromosomal Imbalances in Sporadic Basal Cell Carcinoma by CGH and LOH. *K.J Ashton[1], B.F Williams[2], S.R Weinstein[3], D.J Maguire[4], L.R Griffiths[1].* 1) Genomics Res Ctr, Sch Hlth Sci, Griffith University-Gold Coast, Southport QLD, Australia; 2) Perceptive Scientific International, Chester, United Kingdom; 3) Deparatment of Pathology, Gold Coast Hospital, Southport QLD, Australia; 4) School of BBS, Griffith University-Nathan, Nathan QLD, Australia.

Comparative genomic hybridization (CGH) has become a powerful investigative tool in tumorigenesis allowing the mapping of chromosomal imbalances to normal metaphase spreads. In conjunction with tissue microdissection and degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR), CGH can be used to investigate tumors stored as paraffin embedded tissue. We have used this approach to define chromosomal imbalances present in eleven sporadic basal cell carcinoma (BCC) biopsies. We have shown several losses at 9q, which contains the putative tumor suppressor gene *Patched*. This gene maps to 9q22.3 and has been implicated in Gorlin's syndrome (a familial form of BCC) and several sporadic BCCs. These results were validated by the use of four microsatellite markers mapping across 9q21-qter to determine loss of heterozygosity (LOH). In addition other more novel chromosomal imbalances have been identified by CGH including gains on chromosomes 6, 11, 18 and 20 which were not associated with the 9q loss. This study may have important implications in the genetic characterization of BCC tumorigenesis.

Program Nr: 624 from the 1999 ASHG Annual Meeting

Comprehensive evaluation of HER-2/*neu* gene status in Breast cancer by FISH and Immunohistochemistry (IHC) methodologies. *V.R. Babu, C. Hashimoto, I. Klisak, S. Pham, J.B. Peter.* Specialty Laboratories, Santa Monica, CA.

HER-2/*neu* gene amplification/over expression has prognostic value in breast cancer treatment. The literature reveals discordant results between FISH and IHC detection methodologies. To assess the relative roles played by these techniques in clinical practice, we conducted a retrospective, blind study in which formalin-fixed paraffin-embedded sections from 40 breast cancer patients including six controls were subjected to FISH and IHC testing. FISH was done using PathVysion^{TM} kit (Vysis). If a tumor has an aggregate ratio of equal or greater than 2 between HER-2/*neu* gene copy number and chromosome 17 centromere, it was considered amplified; less than 2 was scored as unamplified. For IHC, Dako Antibody (A0485) was used with the following scoring criteria based on staining intensity: 0, 1+ unamplified; 2+, 3+ amplified. 100% concordance was obtained on all controls. Of the 34 test cases, by both methods, 23 were unamplified and 4 amplified. Seven had discordant results. A scond prospective study of 11 new consecutive cases was undertaken using adjacent tissue sections for IHC and FISH. Tumor cells were identified by H & E stain prior to FISH scoring. Of the 11, seven were unamplified and two amplified by both methods. There were two discordant results. The combined results revealed that 1) there was 100% concordance between FISH and IHC for unamplified patients 2) only 75% of IHC 3+ (6 of 8) positive cases had gene amplification by FISH 3) none (7 of 7) of IHC 2+ positive cases showed gene amplification by FISH. A repeat study with sequential H & E and FISH scoring on one of the patients with discordant results produced the same results. We conclude that IHC is an efficient screening method in the investigation of HER-2/*neu* gene amplification/over expression and FISH is a useful, objective, confirmatory technique for all IHC 'positive' cases. Since only 25% of breast cancers show HER-2/*neu* gene amplification/over expression, the proposed reflex protocol has significant cost benefits to patients. As further methodological enhancements emerge, the proper placement of IHC 2+ and 3+ cases with respect to FISH testing might become clear.

Program Nr: 625 from the 1999 ASHG Annual Meeting

Mutations in the VHL gene as biomarkers of histological discrimination in renal epithelial neoplasia. *N. Barnabas[1], M. Amin[1], K.R Pindolia[1], R. Nanavati[1], M.B. Amin[2], M.J. Worsham[1].* 1) Cancer Genetics Research, Henry Ford Health Systems, Detroit, MI; 2) Emory University, Atlanta, GA.

Renal epithelial neoplasm's (REN) with abundant granular cytoplasm include oncocytomas, eosinophillic variants of chromophobe, papillary renal carcinoma (RCC), collecting duct carcinoma and some conventional (clear cell) RCC. There is controversy whether granular RCC not otherwise specified (NOS) is a distinctive subtype of RCC. To determine if mutations in the VHL gene could serve as ancillary diagnostic markers for histologic subtypes of REN, we analyzed 69 REN-53 carcinomas: 25 clear cell (CC): 18 conventional RCC's (including 4 mixed : clear and granular cells and 14 with clear histology) plus 7 RCC-NOS, 15 chromophobes (CP, including 6 with classic and 9 with eosinophilic histology); 14 papillary (PC), and 16 oncocytomas (OC), benign lesions). Exons 1,2 and 3 of the VHL gene were evaluated using sequencing analysis . Six of the 7 granular RCC-NOS cases had potential mutants in tumors compared to normal tissue, as did 5/14 cases of conventional clear cell carcinoma and 3/4 of mixed call carcinoma. In the CC group, the high frequency of VHL mutations of 60 percent in conventional and granular RCC-NOS suggest that the latter is a histologic variant encountered in conventional RCC and is associated with high-grade histology. Potential mutants were observed in 40 percent of CP patients and were absent in OC and PC. The majority of the these mutations in the CP group were clustered in the eosinophillic subtype (4/9). Involvement of exon 3 was observed exclusively in the CC group. The latter may aid in morphological distinction between CC and CP with clear cells. No mutations were observed in exon 2 in either CC or CP. Similarly, diagnostic differentiation between CP and OC, particularly in the presence of granular cytoplasm, which is problematic at the microscopic level, would be facilitated at the molecular level by VHL shifts if favor of CP over OC. An example of sequencing analysis in one CC (CL13) case revealed an ins G at codon 206 resulting in a frameshift mutation in exon 3. Mutations in the VHL gene may serve as biomarkers of histologic discrimination in REN.

Program Nr: 626 from the 1999 ASHG Annual Meeting

Identification of novel tumor associated antigens in a cohort of patients undergoing an immunotherapeutic clinical trial for advanced stage melanoma. *B.R. Bellows[1], R.M. Conry[2], M.C. Bonnet[3], A.F. LoBuglio[2], T.V. Strong[2].* 1) Department of Human Genetics, University of Alabama at Birmingham, Birmingham, Alabama; 2) Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, Alabama; 3) Cancer Program, Pasteur Meuriex Connaught, Leon, France.

The identification of novel tumor associated antigens may be biologically useful for a number of reasons, including identifying genes important in the progression of cells from normal to the malignant state, identifying targets for cancer detection/diagnosis, providing insight into mechanisms of immune rejection/escape, and expanding targets for immunotherapeutic treatments. A number of these antigenic proteins have been identified, and human clinical observations suggest that immune responses to such antigenic proteins may act to excel tumor destruction through natural defense mechanisms. In this study, a group of 9 stage IV melanoma patients recieved escalating doses of canarypox virus (ALVAC) encoding rhIL-12 intra-tumorally in accessible melanoma nodules. Sera was collected from each patient on day 0, 18, and 43 of treatment. Sera from patient #1, who has experienced a complete response to treatment and has subsequently been disease free for 21 months, was used to screen a cDNA library synthesized from the human melanoma cell lines MEL 624 and MEL 888 in a SEREX strategy. Six immunologically reactive clones have been isolated and partially characterized. These 6 proteins (5 full length proteins and 1 partial sequence) have not been previously implicated as tumor antigens and are not immunologically reactive with a panel of 10 normal sera. Furthermore, 1 of these proteins is reactive with multiple patients, indicating a possible shared antigen. Isotype analysis of the antibody response to these proteins has been established, revealing both IgG1 and IgG3 antibody responses to several proteins. Expression of the genes in the autologous tumor from the reactive patients and in normal tissue is currently being investigated.

Program Nr: 627 from the 1999 ASHG Annual Meeting

Acquired Monosomy 7 in Donor Cells Three Months Post Stem Cell Transplant. *P.K. Berry[1], J.A. Brochstein[3], K. Richkind[2], B. Adler Brecher[3]*. 1) Genzyme Genetics, Yonkers, NY; 2) Genzyme Genetics, Santa Fe, NY; 3) Pediatric Stem Cell Transplant Program, Hackensack University Medical Center.

Three months after unrelated umbilical cord blood transplantation for acute leukemia, a 10 month old baby girl was found to have monosomy 7 in 4 of the 20 male donor cells evaluated by classical cytogenetics. Interphase FISH analysis revealed, in addition to 17% monosomy 7 in donor cells, that 4% of the cells were female. The original donor cells were examined using interphase FISH and were male and negative for monosomy 7. Over the course of the next six weeks there was an increase in monosomy 7 cells, which peaked at approximately 42% in the donor cells. Concurrently the percent of female cells determined by interphase FISH also increased and peaked around 80%. It is interesting to note that interphase FISH consistently detected a higher percentage of female cells than classical cytogenetics. Flow cytometry at this point was most consistent with recurrence of the patient's precursor B-cell ALL and the DNA content showed a diploid pattern with an intermediate S-phase fraction. Chemotherapy was initiated approximately six weeks after the initial identification of aberrant host cells. Subsequently, the number of both the female cells and the monosomy 7 donor cells decreased. Eight weeks later the marrow returned to apparently normal male cells by classical cytogenetics and interphase FISH. Secondary MDS in donor cells is rare, only four cases having been reported in the literature. This is the first case of MDS in donor cells presenting in such a short time post stem cell transplantation and identified in cord blood-derived donor cells. These findings underscore the importance of both classical cytogenetics and FISH studies to monitor the course of bone marrow transplants, both from same sex and opposite sex donors, as well as, those where chromosome markers are present in the recipient cells.

Program Nr: 628 from the 1999 ASHG Annual Meeting

Psychosocial factors predicting genetic testing decisions in members of hereditary breast and ovarian cancer families. *B.B. Biesecker[1], N. Ishibe[2], D.W. Hadley[1], T.R. Giambarresi[2], R.G. Kase[2], C. Lerman[3], J.P. Struewing[4]*. 1) Medical Genetics Branch, NHGRI/NIH, Bethesda, MD; 2) Genetic Epidemiology Branch, NCI/NIH, Bethesda, MD; 3) Lombardi Cancer Center, Georgetown University Medical Cntr Washington DC; 4) Laboratory of Population Genetics, NCI/NIH, Bethesda, MD.

Much is yet to be learned about the most effective ways to provide counseling to persons interested in BRCA1/2 testing. The goal of our study was to identify factors associated with genetic testing decisions in a cohort of hereditary breast and ovarian cancer families. These extended families are known to carry BRCA1 or BRCA2 mutations. Personality traits, family functioning and sociodemographics were self-assessed using psychometric instruments at baseline. Among 172 individuals who participated in pre-test education and counseling, 135 (78 percent) chose to undergo genetic testing and 37 (22 percent) chose not to be tested. Individuals who chose to undergo genetic testing were more likely to be older (>40 yrs), to have higher levels of optimism and to report higher levels of cohesiveness in their families. A better understanding of factors which influence interest in predictive testing may help to inform the counseling that occurs prior to genetic testing.

Program Nr: 629 from the 1999 ASHG Annual Meeting

Low frequency of p53 gene mutations in breast cancers of Japanese-American women. *H. Blaszyk[1], C.H. Buzin[2], S.E. Tang[2], A. Shibata[3], J.M. Cunningham[1], R.K. Ross[4], J.S. Kovach[2]*. 1) Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN; 2) Molecular Medicine, City of Hope, Duarte, CA; 3) Epidemiology and Health Research, Stanford University School of Medicine, Stanford, CA; 4) Preventive Medicine, USC/Norris Comprehensive Cancer Center, Los Angeles, CA.

Differences in frequencies and patterns of somatic p53 gene mutations among racially and geographically diverse populations presumably reflect exposure to different mutagens or different responses to certain mutagens. Upon emigration to the United States, Japanese women experience, over several generations, a 4-5 fold increase in the incidence of breast cancer. To determine whether this increased incidence is associated with a change in the frequency and/or type of p53 gene mutation in their tumors, we examined paraffin-embedded samples of primary breast cancers from Japanese-American women in Los Angeles County, CA. Mutations in exons 5-9 and adjacent intronic regions of the p53 gene were identified and confirmed by direct sequencing. Seven mutations, including 5 missense, were detected in 44 primary breast cancers, a frequency of 16%. There were six transitions and one transversion, including a very rare nonsense mutation at codon 242. As expected, overexpression of P53 protein, detected by immunohistochemistry, occured in tumors with missense mutations; tumors with nonsense or splice junction mutations had no detectable P53 protein. The frequency of p53 gene mutations showed no increase over that previously found in breast cancers of native Japanese and U.S. women. The increased incidence of breast cancer in Japanese-American women is unlikely to be due to a single dominant mutagen and probably multifactorial in nature, warranting further studies.

Program Nr: 630 from the 1999 ASHG Annual Meeting

No association between a (CA)n repeat in the promoter region of the insulin-like growth factor-1 (IGF-1) gene and sporadic prostate and breast cancer cases. *S. Bochum[1], L. Correa-Cerro[1], J. Haeussler[1], O. Cussenot[2], T. Paiss[3], W. Vogel[1]*. 1) Dept. of Medical Genetics, University of Ulm, Germany; 2) Saint Louis Hospital, Paris, France; 3) Dept. of Urology, University of Ulm, Germany.

IGF-1 is a potent mitogen and stimulates growth and inhibits apoptosis of prostate epithelial cells. Recently, several studies suggested that elevated serum IGF-1 levels may be an important predictor of risk for prostate cancer. Chan et al. reported that men in the highest quartile of IGF-1 serum levels had a more than 4 times greater risk developing prostate cancer than men in the lowest quartile, regardless of their baseline level of PSA. Thus, the association between circulating IGF-1 level and risk of prostate cancer seems to be stronger than that of any previously reported risk factor, though there is still some debate. IGF-1 is also implicated in the development and progression of breast cancer and high serum levels have been correlated with breast cancer risk, too. Low IGF-1 serum levels on the other hand are associated with an increased risk for osteoporosis in men. In the latter case a correlation of the IGF-1 serum level and a polymorphic (CA)n repeat in the promoter region about 300 bp upstream the start codon of the IGF-1 gene was shown, indicating functionally different alleles. Because misclassifications of serum IGF-1 levels resulting from variations in measurement accuracy are possible, the detection of a predictive marker on the DNA level could be of great importance for disease prevention and detection. Therefore, we used the polymorphic (CA)n repeat to look for an association in 345 sporadic prostate and in 72 sporadic breast cancer cases and compared them with 182 controls. Neither of these two studies detected any difference in the allele distribution of cases and controls by contingency tables (chi-square-test) or an association between alleles and cancer risk by logistic regression analysis. This result remained unchanged after stratification by age of disease onset or - in the case of prostate cancer - Gleason score. Thus, we conclude that the (CA)n repeat in the IGF-1 gene is not a useful predictor of sporadic prostate or breast cancer at least in the European population.

Program Nr: 631 from the 1999 ASHG Annual Meeting

Positional cloning of a renal cell carcinoma associated familial t(2;3) translocation breakpoint. *D. Bodmer[1], M. Eleveld[1], T. Dijkhuizen[2], I. Janssen[1], M. Weterman[1], H. Janssen[1], A. Geurts van Kessel[1].* 1) Human Genetics, University Hospital Nijmegen, Nijmegen, Gelderland, The Netherlands; 2) Medical Genetics, University of Groningen, Groningen, The Netherlands.

Hereditary renal cell carcinomas (RCC) occur rare and are often reported as a consequence of Von Hippel Lindau (VHL) disease. Also, some RCC families have been encountered exhibiting balanced translocations between chromosome 3 and other chromosomes. In one of these cases, t(3;8), the breakpoint has been cloned and found to occur within a gene on chromosome 3, designated FHIT. Subsequently, it was found that in t(3;8) family members the FHIT gene is fused to another gene on chromosome 8, designated TCR8. The exact role of these (fusion) genes in renal oncogenesis still remains to be established. Recently, we have identified a RCC family with a t(2;3)(q35;q21) chromosome translocation. We have set out to clone the breakpoints involved. To this end, a YAC/PAC/cosmid contig has been established that covers the 3q21 breakpoint region. Currently, we are characterizing breakpoint spanning PACs and cosmids in detail and are searching for gene(s) neighboring and/or spanning the breakpoint. Interestingly, we recently encountered a sporadic RCC case showing the same t(2;3)(q35;q21) as was found in the family. Detailed investigation of tumormaterial using our YAC/PAC/cosmid contig revealed that the 3q21 breakpoint in the sporadic case is not identical to that in the familial case, and is positioned more than 300 kb towards the telomere.

Program Nr: 632 from the 1999 ASHG Annual Meeting

Cytogenetic, molecular cytogenetic, and mutational analysis of 8q24.1 (EXT1) in 47 osteochondroma and chondrosarcoma specimens. *A.K. Boehm, C. Orndal, M. Nelson, E. McComb, J. Neff, R. Robocki, J.A. Bridge.*
Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE.

Osteochondroma most frequently arises sporadically and as a solitary lesion, but may also arise as multiple lesions characterizing the autosomal dominant disorder hereditary multiple exostoses (HME) and the contiguous gene syndromes, DEFECT-11 and Langer-Giedion syndrome (LGS). HME is genetically heterogeneous. Multiple germline mutations of two putative tumor suppressor genes, EXT1 (8q24.1) and EXT2 (11p11-12), have been demonstrated in HME families. In this study, cytogenetic analyses of 47 sporadic and hereditary osteochondroma and chondrosarcoma revealed loss or structural rearrangements of 8q24.1 and 11p11-12 in nearly all clonally abnormal osteochondromas (13/15 tumor specimens). Subsequently, molecular cytogenetic studies were performed on these specimens utilizing an 8q24.1 cosmid probe (locus D8S51, within the minimal LGS deletion region) and a chromosome 8 specific centromeric probe (control for chromosome 8 copy number) to determine the presence and frequency of submicroscopic deletions. Loss of 8q24.1 was found in 20 of 26 (77%) osteochondromas and 13 of 21 (62%) chondrosarcomas. Lastly, these specimens were subjected to sequence analysis studies of exon 6 (the site of a known deletion hotspot in some HME families). No functional mutations of this exon were detected. It is important to note, however, that these studies do not exclude the possible presence of mutations involving other EXT1 exons. In conclusion, these data indicate that a significant subset of sporadic osteochondromas and chondrosarcomas harbor somatic genetic aberrations at the EXT1 locus, and that loss or mutation of EXT1 may be important in the pathogenesis of sporadic as well as hereditary osteochondromas and chondrosarcomas. Additional studies aimed at the examination of other EXT1 and EXT2 exons may reveal an even higher frequency of deletions or functional mutations of these putative tumor suppressor genes in these neoplasms.

Program Nr: 633 from the 1999 ASHG Annual Meeting

Gene variation and cancer risk: generation of complex haplotypes at the *ATM* locus. *P.E. Bonnen, D.L. Nelson.*
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The majority of cancer cases are sporadic, yet it is likely that all tumors carry genetic lesions. Whether genetic variation in the human population leads to differences in cancer predisposition is unknown. The complexity of this question leads to a need for new methods to explore the effects of functional variants of genes and the interactions of multiple genes. We aim to develop haplotypes and perform association studies to determine if haplotypes at specific cancer-associated genes lend increased or decreased risks for cancer. We hypothesize that functional variants of genes involved in familial cancer participate in sporadic cancer. *ATM* is the gene responsible for the autosomal recessive disease ataxia-telangiectasia (AT). AT is characterized by cerebellar ataxia, oculocutaneous telangiectasia, sensitivity to ionizing radiation, increased incidence of tumors, and chromosomal instability. Heterozygotes are at risk for developing cancer, especially breast cancer. The gene spans 184 kb and contains 66 exons. We used gel-based re-sequencing to examine 29 regions (a total of 13.5 kb) across the *ATM* gene in five unrelated individuals of varied ethnic origin. We detected 17 single nucleotide polymorphisms (SNPs) over these regions. We then used allele specific oligonucleotide (ASO) hybridization assays to determine the genotypes of 9 CEPH families (87 individuals) and 266 samples from four different ethnic origins. Analysis of these data with the program EMHAPFRE has allowed definition of haplotypes and their frequencies. Our next aim is to determine the prevalence of these haplotypes in populations such as AT, breast cancer, and appropriate controls; haplotypes showing an association can be further investigated to determine their contributory functional variation. This study is part of a larger project that aims to develop haplotypes for numerous other cancer-associated genes (e.g. *p53* and *BRCA1*)and to examine the potential combined effects of these genes with *ATM*.

Program Nr: 634 from the 1999 ASHG Annual Meeting

Tracking BMT Patients Using FISH on Flow-sorted Cell Populations. *L.J. Brothman[1], Z. Chen[1], R.H. Adams[1], W.F. Green[3], X.L. Zhu[1], A.R. Brothman[1,2].* 1) Dept. of Pediatrics; 2) Dept. Hum. Genet; 3) Dept. of Int. Med., Univ of Utah, Salt Lake City, UT.

A test was devised to study different cell populations in patients following sex-mismatched bone marrow transplant (BMT). Populations of granulocytes, monocytes, T, B, NK and CD34+ cells were sorted directly onto microscope slides using a BD FACSVantage flow cytometer equipped with an automatic cell deposition unit, with 500 cells/spot. Slides were dried and fixed with 3:1 MeOH:acetic acid prior to FISH. FISH with an X a- and Y-classical satellite probe (DXZ1, DYZ1, Oncor or Vysis) was performed on each of the cell populations. Two patients were initially evaluated and 5-7 sorts each were done following BMT. Patient #450, male, was diagnosed in utero with infantile metachromatic leukodystrophy and underwent an unrelated BMT at 3 mo. of age. Evaluation at day 100 revealed autologous BM reconstitution, persistent abnormal levels of aryl sulfatase A and a 46,XY host karyotype. At 7 mo., a second unrelated BMT was done, and cytogenetics performed 1 mo. later showed XX and XY cells. Initial evaluation of sorted cells showed the T-cells were primarily donor in origin, but NK cells and granulocytes were primarily of host origin. Because of the apparent partial engraftment, immunosuppression was stopped and the patient was monitored clinically. Additional FAC-sorted FISH demonstrated progressive donor engraftment of all cell lines tested. Patient #337, male, was diagnosed with Wiskott-Aldrich Syndrome at 6 mo. of age and underwent HLA-matched cord blood transplant at 18 mo. from his female sibling. BM cytogenetic evaluation at 100 days post BMT revealed 73% XY host cells and 27%XX cells. Cells were sorted to identify lineage origin; B-cells and granulocytes were >50% XY, while T-cells were nearly all XX (95%). He was tapered off immunosuppression and his pattern of engraftment followed with additional sorted samples. FAC sorted FISH analysis can be an invaluable tool for quick and accurate definition of cell line engraftment in patients who have a mixed chimeric pattern of engraftment following BMT. This information can be a useful guide to immunosuppression and clinical management.

Program Nr: 635 from the 1999 ASHG Annual Meeting

Quality Control in Haematological diagnosis through Cytogenetics and Molecular study in Patient with suspicion of LEUKEMIA Ph+. *R. Burgos[1], P.E. Leone[1,2], J.C. Pérez[1], V. Dávalos[1], M.E. Sánchez[1,2], J.C. Santos[1], C. Paz-y-Mino[1,2]*. 1) Hum Mol Genet & Cytogen Lab., Ecuadorian Catholic University, Quito, Ecuador; 2) Medicine Faculty. Ecuadorian Catholic University.

Cytogenetic and molecular studies are employed to diagnosis of leukemia and serve in order to improve the diagnosis and could be used as a quality control for haematological diagnosis. One of the most characteristic alterations in leukemia are the Philadelphia chromosome (Ph+), which is product of translocation between 9 and 22 chromosomes t(9;22) (q34; q11), affecting the BCR-ABL oncogene. This aberration is presented in 95% of individuals with LMC and approximately 15% with LLA. We analyzed 75 individuals that presented diagnosis for leukemia; 35 with Chronic Myeloid Leukemia (CML) and 40 with Acute Lymphoblastic Leukemia (ALL). All individuals were studied for cytogenetic and molecular level using Reverse Transcription Polymerase Chain Reaction (RT-PCR), which has detected 37.5%(ALL) and 62.8%(CML) of all cases that present the re-arrangement t(9; 22) (q34; q11), BCR-ABL oncogene. This data suggests that exists a high percentage of individuals with erroneous diagnosis in Ecuador, according to what was proved in haematological reevaluation of previous diagnosis. We suggest the necessity to apply both of techniques in order to discard or confirm the presumed diagnosis and increase specificity and sensibility of diagnosis. This study is supported by BID-Fundacyt Project.

Program Nr: 636 from the 1999 ASHG Annual Meeting

A minimally invasive assay detects a BRCA2 germline mutation. *T.J. Byrne, M.T. Reece, M.A. Lane, L. Adams, G.M. Cohn.* Ob/Gyn Research, Baystate Medical Center, Springfield, MA.

Many individuals at risk for heritable BRCA1 or BRCA2 mutations may not receive appropriate screening and/or treatment without a definitive molecular diagnosis. Currently, clinical genetic testing requires gene sequencing which is expensive and time consuming. Earlier, we reported the development of an antibody-based assay that correlated with BRCA1 gene alterations in surgical specimens. Subsequently, this assay applied to buccal cells was predictive of BRCA1 gene mutations among at risk individuals. Here, we present a similar assay for BRCA2. Immunoreactivity for antibodies directed against the BRCA2 carboxy and amino terminal groups was observed in buccal cells of low risk controls. A reduction in carboxy group reactivity, relative to amino group reactivity, was noted in the buccal cells of an individual found to have a BRCA2 germline mutation. These results suggest that a simple, antibody-based assay, may serve as a rapid, minimally invasive and inexpensive clinical tool for detecting BRCA1 and BRCA2 mutations that result in protein trunctions.

Program Nr: 637 from the 1999 ASHG Annual Meeting

Characterization of a Carboxypeptidase-A Inhibitor Identified by DD-PCR in Primary Ovarian Tumors and Cell Lines. *G. Callahan, V. Shridhar, L. Hartmann, D.I. Smith.* Experimental Pathology, Mayo Foundation, Rochester, MN.

One in seventy American women will be diagnosed with ovarian cancer and one in a hundred will succumb to the disease. This high mortality rate dictates the necessity for earlier detection and prevention. In order to find genes related to the development of ovarian cancer, we performed differential display PCR analysis on short-term culture, normal ovarian epithelial cells versus four low-passage ovarian cancer cell lines established at Mayo and OVCAR5. We have isolated an interesting candidate gene, which is highly expressed in normal ovarian epithelial cells, but completely absent in 2 of the 5 tested tumor-derived cell lines. Sequencing followed by EST-based walking allowed us to generate the open reading frame for this gene. It codes for a protein that is 222 amino acids long with 85% identity to the mouse latexin protein. In mouse and rat, latexin has been shown to be an inhibitor of carboxypeptidase A, and it contains two potential Ca^{2+}/calmodulin-dependent protein kinase sites and one c-GMP-dependent protein kinase phosphorylation site. On Northern blots, a 2kb transcript was observed at very high levels in normal ovarian epithelium with absence of expression in 2 of 5 ovarian cancer cell lines and 6 of 12 primary ovarian tumors, including 4 out of 8 early stage tumors. In addition, we were able to induce expression in a dose-dependent manner in OV-202 upon 5-aza-2'-deoxycytidine treatment, implicating methylation as one of the mechanisms for inactivation of this gene. Using the Stanford G3 radiation hybrid mapping panel, we localized this gene to human chromosome 3q25. Transfection experiments are currently in progress to examine the gene's functional relevance to ovarian cancer progression.

Program Nr: 638 from the 1999 ASHG Annual Meeting

Lung cancer risk associated with family histories of smoking and lung cancer. *C.L. Carpenter[1], S.J. London[2]*. 1) Preventive Medicine, USC/Norris Compr Cncr, Los Angeles, CA; 2) National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Genetic susceptibility to smoking may increase lung cancer risk because of increasing the likelihood of heavier smoking patterns and greater difficulty in quitting. Family history, often used as a surrogate estimator of genetic susceptibility, has been studied extensively in relationship to disease susceptibility but not in relationship to exposure susceptibility. To assess the lung cancer risk of family history of smoking and family history of lung cancer, we conducted analyses from a population-based case-control study of lung cancer in African American and Caucasian residents of Los Angeles County who ranged in age from 40 to 85 years. In-person interviews elicited smoking history, dietary patterns, occupational exposure and information about other related covariates. Study participants were asked about histories of lung, other cancers and smoking patterns for each of their primary family members (parents, siblings and children). Unconditional logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI). Analyses were restricted to 354 cases and 721 controls that had complete information on smoking and family history. Eleven percent of study participants (14% cases, 10% controls) had a positive family history of lung cancer and 88% of participants (94% cases, 86% controls) had a positive family history of smoking.

Family history of lung cancer was moderately associated with increased risk of lung cancer (OR=1.63; 95% CI = 1.02-2.60), adjusting for family history of smoking, age, race, gender, total pack-years and years since quitting. Family history of smoking was also moderately associated with lung cancer risk (OR=1.78; 95% CI=1.02-3.11), adjusting for family history of lung cancer and study covariates.

Our results suggest that family history of lung cancer and family history of smoking may have independent effects on lung cancer risk.

Program Nr: 639 from the 1999 ASHG Annual Meeting

Absence of germline MSH6 mutations in hereditary nonpolyposis colorectal cancer (HNPCC) and endometrial cancer kindreds. *R.B. Chadwick[1,2], T.W. Prior[2], R. Pyatt[2], C.K. Johnson[1], T.H. Niemann[2], H. Hampel[1], J.S. Graham[1], A. de la Chapelle[1]*. 1) Division of Human Cancer Genetics, Ohio State University, Columbus, OH; 2) Department of Pathology, Ohio State University, Columbus, OH.

Inherited mutations in genes involved in DNA repair lead to early development of colorectal cancer. The primary genes which have been implicated in the familial cancer syndrome known as hereditary nonpolyposis colorectal cancer (HNPCC) are the MSH2 and MLH1 genes. In HNPCC kindreds there is also a greater susceptibility to endometrial cancer. The protein products of the MSH2 and MLH1 genes recognize DNA mismatches and form complexes with each other and with the PMS1, PMS2 and MSH6 proteins. Loss of DNA repair function due to mutation and genomic deletion of both copies of the MSH2 or MLH1 genes leads to a mutator phenotype and tumors which develop in these individuals display microsatellite instability (MSI). There has been a recent report of hereditary mutations being found at a high rate in the MSH6 gene and that tumors which develop in these individuals are not MSI positive or are weakly MSI positive. This study was undertaken in order to determine the mutational frequency of the MSH6 gene in both MSI positive and MSI negative colorectal and endometrial cancers. Individuals from ten MSI positive and five MSI negative HNPCC families were screened for mutations in the MSH6 gene by direct sequencing of all ten exons and flanking intronic regions. Using the same method individuals from ten MSI negative and 14 MSI positive endometrial cancer families were screened for MSH6 mutations. In DNA isolated from blood, only minor amino acid changes were found in the MSH6 gene which are unlikely to be pathogenic. However, in DNA isolated from tumors somatic pathogenic truncating mutations were found. Hence, this study suggests that germline mutation in the MSH6 gene is not a frequent cause of predisposition to familial colorectal and endometrial cancers, but somatic mutations may play a role in the transformation process of cancers in these organs.

Program Nr: 640 from the 1999 ASHG Annual Meeting

Germline polymorphisms in the methylenetetrahydrofolate reductase (MTHFR) and cytochrome-P450 1A1 (CYP1A1) genes and B-cell neoplasm susceptibility. *S.O. Chan, S.L. Gersen.* DIANON Systems, Inc, Stratford, CT.

A recent study indicated that germline polymorphisms, C667T and C4887T, in the MTHFR and CYP1A1 genes, respectively increase the susceptibility of women to endometrial cancer. The association of these polymorphisms with B-cell neoplasms was investigated. Allele specific PCR was used to determine genotypes. Genotype frequency of the MTHFR polymorphism was established for 91 patients with various B-cell neoplasms and was compared with the frequency in 186 controls. No significant association between the C667T polymorphism and risk of B-cell neoplasm was found, with an odds ratio of 0.97 (95% confidence interval: 0.64-1.49, P=0.8936). The frequency of the C4887T polymorphism was determined in 137 patients and was compared with the frequency in 100 controls. A significant association between the C4887T polymorphism and risk of B-cell neoplasm was found, with an odds ratio of 10.38 (95% confidence interval: 1.34-80.74, P=0.0062). Thus, this study suggests that the C4887T polymorphism in the cytochrome-P450 1A1 gene may influence susceptibility to B-cell neoplasms. Larger sample sizes are required to corroborate these findings.

Program Nr: 641 from the 1999 ASHG Annual Meeting

A Functional Genetic Strategy to Identify Novel Genes in the Chromosome 3p12-Mediated Pathway to Tumorigenesis in the Kidney. *D.S. Chandler, S.T. Lott, D.L. Tirpak, A.M. Killary.* Department of Pathology and Laboratory Medicine, UT M.D. Anderson Cancer Center, Houston, TX.

High frequency loss of heterozygosity, homozygous deletion, cytogenetic aberrations and our own physical mapping and functional complementation studies have implicated chromosome 3p12 to contain an important tumor suppressor gene for renal cell carcinoma. Our laboratory has taken a functional genetic strategy to identify novel genes involved in the chromosome 3p pathway to tumorigenesis in the kidney. Defined microcell hybrid clones were previously constructed by the transfer of a 3p centric fragment encompassing 3p12-q11 into three different RCC cell backgrounds. Transfer of this 3p fragment resulted in dramatic tumor suppression concomitant with rapid cell death of RCC cells *in vivo* and allowed the physical mapping of a novel tumor suppressor locus *NRC-1* into the 3p12 interval. We now report the utilization of these hybrid clones, differing by only 1-2 Mb within 3p12 and yet phenotypically differing greatly in their ability to form progressive tumors in athymic nude mice, as starting materials to identify downstream genes in the 3p12 pathway to tumorigenesis. PCR select subtractive hybridization was used to identify cDNAs that were differentially expressed between microcell hybrids suppressed or unsuppressed for tumorigenecity *in vivo*. Nine hundred partial cDNA clones were identified using PCR select subtraction. Nine of ten cDNAs selected at random were sequenced and found to be novel cDNAs with sequence similarities including genes involved in transcriptional regulation and cell adhesion. In addition, RNase protection assays identified cDNAs that were expressed exclusively in hybrids suppressed for tumorigenicity and absent or downregulated for expression in RCC parental cell lines. We are currently screening for differential expression of PCR-select clones using microarray technology and RNase protection assays. The long term goal of these studies will be the definition of genetic pathways to tumorigenesis in the kidney.

Program Nr: 642 from the 1999 ASHG Annual Meeting

Bcl 10 is not commonly mutated in multiple primary solid tumors. *J.G. Chang[1], H.H. Lee[1], N.M. Wang[1], K.T. Yeh[2], F.J. Tsai[1], C.H. Tsai[1]*. 1) Dept Medical Res,Div Molec Med, China Medical Col Hosp, Taichung, Taiwan; 2) Department of Pathology, Changhua Christian Hospital, Changhua.

The Bcl 10 gene was recently discovered to be involved in the pathogenesis of lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) and several types of tumorous cell lines. We examined the Bcl 10 gene in a panel of cancers along with their corresponding non-tumorous tissue by PCR-SSCP and direct sequencing. A total of 8 different types of carcinoma and their paired non-tumorous tissues were studied; and it included 33 breast cancers, 39 gastric cancers, 30 cervical cancers, 31 thyroid cancers, 33 oral cancers, 47 lung cancers, 34 hepatoma and 30 colo-rectal cancers. There were three gene alterations found in this screening: (1) a CTG to CTC change at codon 8 which did not alter the amino acid sequence, (2) a GCA to TCA mutation at codon 5 which changes the amino acid alanine to serine, and (3) a GGA to GAA mutation at codon 213 which changes glycine to glutamic acid. No other gene alteration was detected in the cancerous tissues. However, these mutations were also found in the paired non-tumor tissues and normal individuals' tissues. Therefore, these three changes may be the polymorphic forms of the gene and have no relationship with pathogenesis of malignancy. We suggest that the Bcl 10 gene is rarely involved in the pathogenesis for most types of cancers, and the changes in the tumor cell lines, such as p16 mutation in most of cell line, which may be due to in vitro selection.

Program Nr: 643 from the 1999 ASHG Annual Meeting

Increased incidence of thyroid cancer in first-degree relatives of probands with non-medullary thyroid cancer: a case-control-cohort study. *P.O. Chappuis[1,2], H. Renard[3], T. Kantemiroff[1], K. Sanders[1], A. MacMillan[1], M.J. Black[2], D.E. Goldgar[3], W.D. Foulkes[1,2].* 1) Depts Medicine and Human Genetics, Montreal General Hospital, McGill University, Montreal, QC, Canada; 2) Cancer Prevention Research Unit and Dept. Head and Neck Surgery, SMBD-Jewish General Hospital, McGill University, Montreal, QC, Canada; 3) Unit of Genetic Epidemiology, IARC, F-69372, Lyon, Cedex 08, France.

Non-medullary thyroid cancer (NMTC) is uncommon (~1.5% of all cancers in Canada) but its incidence in Canada is increasing, especially in women (6% increase per year 1987-1996, P=0.01). The heritable fraction of NMTC is uncertain, but families with several cases of NMTC +/- goitre have been reported, and 2 loci (MNG1 and TOC) have been identified. We conducted a case-control-cohort study of 176 prevalent cases (66% of all eligible cases) of NMTC diagnosed 1986-1996 at McGill University teaching hospitals and 198 hospital-based age, sex and ethnically-matched controls. Family history of cancer and thyroid disease was recorded for all first-degree relatives (FDR) of cases and controls. 70.5% of the cases and 72.2% of the controls were females. The ages at interview of cases and controls did not differ (means 48.8 yrs and 48.9 yrs respectively). There were 1157 FDR of cases and 1233 FDR of controls. The mean ages of case and control relatives was 46.9 yrs and 50.2 yrs respectively. There were 10 reported thyroid cancers in case relatives and 2 in control relatives. In a survival analysis, the case and control relatives curves were significantly different (P=0.009, log-rank). The incident rate ratio (IRR) was 5.7 (95%CI: 1.2-53.4, P=0.006). Of note, prostate cancer was also in excess (IRR=3.0, P=0.03). No other cancer site was significantly over-represented. For thyroid cancer, there was no difference in incidence ratio according to the sex of the proband, but the increased risk in case relatives was largely confined to female relatives (IRR 8.7, P=0.008). In a Cox proportional hazards model, the estimated hazard ratio was 6.0 (1.3-27.3, P=0.021). These data show that NMTC has a moderate but real familial component. The underlying genetic basis awaits elucidation.

Program Nr: 644 from the 1999 ASHG Annual Meeting

Hypermethylation at an upstream STAT-binding site inhibits STAT-mediated p21/WAF1 induction in human rhabdomyosarcomas. *B. Chen[1,2], L. He[1], V.H. Savell[1,2], J.J. Jenkins[3], D.M. Parham[1,2]*. 1) Department of Pathology, University of Arkansas for Medical Sciences, Little Rock, AR; 2) Arkansas Children's Hospital, Little Rock, AR; 3) Department of Pathology and Laboratory Medicine, St. Jude Children's Research Hospital, Memphis, TN.

p21/WAF1 functions as a negative regulator of cell proliferation by inhibiting cyclin-dependent kinase activity and blocking the G1-S transition. Levels of p21/WAF1 are often abnormally low in human rhabdomyosarcomas, which resemble fetal muscle but fail to undergo growth arrest or to differentiate. To understand the cause of decreased p21/WAF1 expression in this cancer, we examined the methylation pattern of the p21/WAF1 promoter region in primary rhabdomyosarcomas and rhabdomyosarcoma cell lines. Hypermethylation at a STAT (signal transducers and activators of transcription)-responsive element, SIE-1, was detected in 13 of 26 (50%) primary rhabdomyosarcomas and 2 of 5 rhabdomyosarcoma cell lines. All primary tumors with hypermethylation at SIE-1 showed decreased p21/WAF1 expression compared to normal skeletal muscle. The SIE-1 element is a binding site of STAT proteins, which mediate induction of p21/WAF1 expression following activation by a variety of ligands such as interferon-gamma. However, in rhabdomyosarcoma cell lines hypermethylated at SIE-1, activated STAT proteins failed to induce p21/WAF1 expression, and cells continued to proliferate in the presence of interferon-gamma. In vitro DNA-protein binding experiments indicated that CpG methylation at SIE-1 significantly reduced STAT binding. Furthermore, CpG methylation of luciferase reporter constructs containing SIE-1 significantly inhibited the reporter gene activity and the induction in response to interferon-gamma. These results indicate that hypermethylation at SIE-1 inhibits STAT-mediated up-regulation of p21/WAF1 expression through directly blocking protein-DNA binding. Our findings suggest a new mechanism by which aberrant DNA methylation results in reduced signal transduction and deficient cell cycle regulation in rhabdomyosarcomas.

Program Nr: 645 from the 1999 ASHG Annual Meeting

Mutational analysis of the hSNF5/INI1 gene in rhabdoid tumor. *T.T.-L. Chen, J. Savla, C.F. Timmons, N.R. Schneider, G.E. Tomlinson.* Univ. Texas Southwestern Med., Dallas, TX.

Rhabdoid tumors are extremely aggressive malignant tumors of the kidney which occur in early infancy. Approximately 10-15% of renal rhabdoids are associated with central nervous system (CNS) tumors of various histologies. Monosomy 22 has been reported as a karyotypic finding in some rhabdoid tumors. The hSNF/INI1 gene localized to chromosome 22q11.2 has been reported to be mutated in primary rhabdoid tumors. Using single strand conformation polymorphism analysis, we studied tumor and normal DNA from 4 children who with renal rhabdoid tumors, two of whom also developed a second primary tumor of the CNS. The full open reading frame of the hSNF/INI1 gene was analyzed using primers derived from the exon-intron junctions. Abnormal bands were sequenced to determine mutations. Truncating mutations in germline DNA at codons 91 and 198 occurred in the patients with both renal and CNS primary site tumors. In each case, loss of the normal allele was observed in the tumor tissue. In each of the CNS tumors, the loss of one copy of chromosome 22 was observed. In one of the renal rhabdoid tumors, a second mutation of the hSNF/INI gene was acquired; in the other renal rhabdoid, a loss of the normal allele was observed. We also analyzed germline and tumor DNA from 2 patients with renal rhabdoid tumors who did not develop second primaries tumors of the CNS. In one of these we found an acquired termination mutation at codon 47 which was not present in the germline DNA. We have thus found that the hSNF5/INI1 gene plays an important role in rhabdoid tumors. The observed germline and acquired mutations support the concept that the hSNF5/INI gene acts as a tumor suppressor gene, mutation of which may account for the predisposition to separate primary tumors in the subset of rhabdoid tumor patients who develop both renal and CNS tumors.

hSNF5/INI1.

Program Nr: 646 from the 1999 ASHG Annual Meeting

A de novo DNA sequence related to the metastasis of colorectal cancer. *S.-D. Cheng.* Dept Anatomy, Chang Gung Univ, Col Medicine, Kweishan, Taiwan, R. O. C.

A DNA sequence, temporally named UK1, which is partially homologous to the cDNA of NM23H1 gene, was obtained by screening human genomic lambda library. It was localized at chromosome 7p12 with fluorescence in situ hybridization (FISH). With FISH it was further examined on the interphase nuclei of the colorectal cells. The cells were from the short term primary culture of colorectal cancer tissues, and the patients' normal colon tissues are as control. The Dukes' stage differentiates the various grades of the colorectal cancer depending on the area invaded by the tumor. Of stages A and B tumor cells do not invade lymph nodes. Either stage represents for the nonmetastasis of tumor, with more than 80.4+/-5.6% of nuclei have two copies of the FISH signals indicating the nondeletion of the UK1 sequence (n=24). On the other way, the stage C with lymph nodes invaded and stage D with distant organ(s) invaded represent for the metastasis of tumor cells (n=27). Among the examined nuclei only 66.0+/-6.6% is not deleted of the FISH signals. The control group with the normal tissues from thirteen cases of various stages show at least 94.3+/-0.7% of nuclei have two copies of the FISH signals indicating the hybridization efficiency of the probe. Currently, we have subcloned the EcoRI fragments of UK1 lambda probe into plasmid Bluescript SK(+), and are acquiring their DNA sequences. The goal of this study is pursuing the prospective gene UK1, which maybe relate to the metastasis of colorectal cancer cells.

Program Nr: 647 from the 1999 ASHG Annual Meeting

Gains and losses of chromosomal regions in prostate cancer cell lines with varying metastatic potential as detected by comparative genomic hybridization. *L.W. Chu, C.A. Pettaway, S. Pathak, J.C. Liang.* U.T.M.D. Anderson Cancer Center, Houston, TX.

Recently, Pettaway et al. [1] established an *in vivo* tumor progression model in which subclones of the PC3M and LNCaP cell lines were selected for varying metastatic potential after successive orthotopic implantation in the prostate of nude mice. PC3M and selected variants, PC3M-Pro4 and PC3M-LN4, represent aggressive androgen-independent tumors which can metastasize. However, PC3M-LN4 had a greater incidence of distant metastases. Orthotopic injection of LNCaP, an androgen-dependent cell line, resulted in LNCaP-Pro5 line that is slightly metastatic and LNCaP-LN3 line that readily metastasizes to the lymph nodes and liver after intrasplenic inoculation. In this study, we used comparative genomic hybridization (CGH) to compare the chromosomal abnormalities between the parental cell lines and their respective variants. PC3M showed gains of 8q22-qter, 10q11-q22, 17q22-q24, 18q23, 20q13, and Xq27-qter and losses of 13q34, 17p13, and Y. The two sublines, PC3M-Pro4 and PC3M-LN4, retained most of these aberrations but showed an additional gain of 19q. Furthermore, PC3M-Pro4 had unique gains on 1q32 and 3q13 while PC3M-LN4 had specific gains on 1q21-22 and 18p12-21 suggesting these abnormalities may be important for the different metastatic potential of these two sublines. CGH analysis of LNCaP showed a gain on 3q28-qter and losses on 13q21-qter and Y. In addition to all the abnormalities of the parental LNCaP line, LNCaP-Pro5 and LNCaP-LN3 shared a gain of whole chromosome 3 and losses on 6p21-q16 and 13q14. Moreover, LNCaP-Pro5 had a unique gain on 13q12-q13 and LNCaP-LN3 had specific losses on 16q23-qter and 21q22. Interestingly, regions of loss detected in these cell lines correlated well with LOH at 6q21-q22, 13q14, 16q23-qter, and 21q22 in prostate tumors [2]. Our data suggests that CGH can detect unique abnormalities which distinguish tumors of differing metastatic potential.

[1] Pettaway CA, et al. Clinical Cancer Research. 2: 1627-36, 1996.

[2] Saric T, et al. International Journal of Cancer. 81: 219-24, 1999.

Program Nr: 648 from the 1999 ASHG Annual Meeting

DNA replication arrest in the xeroderma pigmentosum variant following ultraviolet irradiation involves double-strand breaks and recombination, mediated by the hMre11-hRad50-NBS1 complex. *J.E. Cleaver[1, 2], C.L. Limoli[3].*
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Xeroderma pigmentosum (XP) is an autosomal recessive DNA repair disorder characterized by UV sensitivity and a genetic predisposition to solar induced skin cancer. Complementation groups XPA-G exhibit defects in nucleotide excision repair (NER) while the variant (XPV) exhibits defective translesion postreplication repair, but retains otherwise normal NER. To delineate the molecular nature of the bypass replication defect in XPV, SV40 transformed human fibroblasts were exposed to UV light and examined for the appearance of nuclear foci by fluorescence immunolocalization using a polyclonal antibody specific to hMre11. Mammalian Mre11 exists as a multiprotein complex with Rad50 and NBS1, involved in the recombinational repair DNA double-strand breaks (DSB). Normal, XPA and XPV cells all show X-ray inducible Mre11-Rad50-NBS1 foci (~ 25-50% foci positive cells after 6 Gy), but normal and XPA cells do not show any UVC-inducible foci. XPV cells however, show foci positive cells following UVC exposure (~ 10-15% after 12 Jm-2). Colocalization experiments suggest that the Mre11-Rad50-NBS1 foci seen after UV irradiation in XPV cells are distinct from repair complexes containing the Rad51 protein. Antibodies specific to BrdU and PCNA indicate a fraction of the UV-induced foci in XPV cells are present in replicating cells. Thus, the formation of UV-induced Mre11-Rad50-NBS1 nuclear foci constitutes a distinguishing feature of the XPV versus other XP cells. The inability to bypass bulky DNA adducts during replication leads to DNA intermediates recognized by the Mre11-Rad50-NBS1 complex, which then mediate their resolution by strand exchanges involving the transient formation of DNA DSBs. Work support by NIH grants 1RO1-CA73924 (C.L.L) and 1RO1-CA8061 (JEC).

Program Nr: 649 from the 1999 ASHG Annual Meeting

Mutational ananlysis of candidate breast tumour suppressor genes located at 16q24.3. *A.M. Cleton-Jansen[1,2], E.W. Moerland[1], H.M. Van Beerendonk[1], A. Savoia[3], D.F. Callen[4], G.J.B. Van Ommen[2], C.J. Cornelisse[1]*. 1) Department of Pathology, Leiden University Medical Centre, Leiden, The Netherlands; 2) Department of Human Genetics, Leiden University Medical Centre, Leiden; 3) Servizio di Genetica Medica, IRCCS-Ospedale CSS San Giovanni Rotondo, Italy; 4) Dep. Cytogenetics and Molecular Genetics, Adelaide Women's and Children's Hospital, Australia.

The long arm of chromosome 16 is involved in loss of heterozygosity (LOH) in more than 50% of breast tumours. This strongly suggests the presence of one or more tumour suppressor genes (TSG) on 16q. Proof for its TSG function is the repeated identification of inactivating mutations in the retained copy of a gene in tumours with LOH. Dense LOH mapping with microsatellite markers identified a region at the most distal part of 16q as smallest region of overlap, i.e. 16q24.3. As part of an international collaborative effort a physical and transcription map was constructed to find candidate TSGs. The region with 7 mapped genes comprises 750 kb and as a result of the constructed map at least 20 new genes could be identified. From this total of 27 genes the most likely candidates were screened for mutations. Prior to mutation screening the exon-intron boundaries were determined to be able to design primers for analysis of genomic DNA. Although not 100% efficient the single strand conformation polymorphism (SSCP) analysis was selected as the preferred system for mutation screening because this method is easily set up, which is a prerequisite when a number of different genes must be tested. In addition RT-PCR products were screened for splice mutants and Southern blot analysis was applied to identify possible large genomic deletions. We screened BBC1, FAA, GAS11, PISSLRE, MC1R, SPG7, Copine VII and 3 other transcripts with unknown function in a panel of 15-20 breast tumours with LOH restricted to 16q24 to ensure that a gene at this region is target for LOH and not another TSG at 16q. None of these genes showed tumour restricted alterations. In conclusion, we have excluded 10 genes mapping to the candidate region at 16q24.3 as TSG. The real target of LOH at this region remains to be identified.

Program Nr: 650 from the 1999 ASHG Annual Meeting

Identification of consistent aberrations in myxopapillary ependymoma tumours. *N. Coleman[1], D. Sanoudou[1], O. Tingby[1], M.A. Ferguson-Smith[2], V.P. Collins[1].* 1) Dept Pathology, University of Cambridge, Cambridge, England; 2) Dept Veterinary Medicine, University of Cambridge, Cambridge, England.

Myxopapillary ependymomas (ME) are a benign variant of ependymomas, usually located at the cauda equina. They have a tendency for slow growth and local recurrence without progression. They may also spread within the nervous system and occasionally metastasise extraneurally. The overall survival depends on the extent of surgical removal of the tumour. Previous studies agree on the rare immunocytochemical identification of p53 expression and the low proliferative activity. The presence of the bcl-2 oncoprotein has also been shown immunohistochemically. Limited cytogenetic analysis of rare ME tumours cell cultures has not demonstrated any consistent chromosomal abnormality. In the present study frozen tissue from seven ME patients was analysed by comparative genomic hybridisation (CGH), microsatellite markers and interphase fluorescent in situ hybridisation (FISH). All seven cases had gain of chromosomes 9 and 18. Chromosome 9 was present in either three or four copies. Chromosome 18 was present in four copies, which appear to arise from the duplication of both chromosome 18 alleles. Other aberrations included gains on chromosomes 3, 4, 5, 7, 8, 13, 17, 20 and X, and losses on chromosomes 10 and 22. These findings represent the first steps towards understanding the molecular mechanisms involved in the development of this tumour form.

Program Nr: 651 from the 1999 ASHG Annual Meeting

Microsatellite Instability (MSI) *In Vitro* vs.*In Vivo*? *M.I. Coolbaugh-Murphy[1], A. Maleki[1], L.C. Strong[2], P.M. Lynch[3], M. Frazier[3], D.G. Monckton[5], B.W. Brown[4], M.J. Siciliano[1]*. 1) Molecular Genetics; 2) Experimental Pediatrics; 3) GI Oncology; 4) Biomathematics,UT-MD Anderson Cancer Center, Houston, TX; 5) Molecular Genetics, Univ. of Glasgow, Glasgow, U.K.

MSI, associated with several types of cancer, is seen by PCR detection of new alleles. Variant fragments need to be well represented in the tumor to be so detected. For detection and quantitation of rare variant fragments, small pool PCR (SP-PCR) may be done. SP-PCR uses DNA diluted down to less than 1ge. Analysis of many small pools can identify infrequent fragments which would be hidden by standard PCR procedures. Conducting such analyses on HNPCC and LFS DNA, at the usually stable DM locus, suggested that as ge. decreased from 10 ge. to <1 ge., the frequency of detection of unobserved variants increased. Is this due to true detection of infrequent mutants or PCR artifact generation as ge. decreases? Therefore, PCRs were conducted on multiple 1ge's of HNPCC tumor DNA and after the 5 cycles, 1/2 of each reaction mixture was removed and set aside (aliquot B). Each remaining portion (aliquot A) completed the nested PCR analysis. If a variant was seen in any of those pools, its (aliquot B) was diluted to single molecules and nested PCR conducted on each. Recovery of the variant seen in (aliquot A) indicated that it was present in the original DNA or generated in one of the first five rounds of amplification. Any fragment seen after expansion of (aliquot B) that was not seen in (aliquot A), must be PCR artifact. We report here that the artifact frequency is 0.005 as determined by this procedure. Thus artifact is not seen as a factor in MSI. To determine the optimal ge. level for accurately detecting and quantitating mutation frequency, reconstruction experiments were conducted. DNAs of two heterozygotes with different alleles at the DM locus were mixed together in various proportions. We then identified the ge. level at which the less frequent alleles remain detectable at the expected frequency. The upper limit for optimum allele detection was <5 ge. Whereas, optimal frequency determination and isolation of alleles occurs at 0.8 ge.(1.6 alleles) per reaction. *In Vitro*.

Program Nr: 652 from the 1999 ASHG Annual Meeting

Linkage analysis of 153 prostate cancer families over a 30 cM region containing the putative susceptibility locus HPCX. *K.A. Cooney[1], H. Chen[1], M.E. Ray[1], K. Brierley[1], E. Perrone[1], C.H. Bock[1], E. Gillanders[2], E.M. Lange[1]*. 1) Departments of Internal Medicine, Surgery, Radiation Oncology and Biostatistics, University of Michigan, Ann Arbor, MI; 2) Cancer Genetics Branch, National Human Genome Research Institute, Bethesda, MD.

Epidemiological studies have provided data to support the hypothesis that there are genes on the X chromosome that may contribute to prostate cancer susceptibility. A recent linkage study of 360 prostate cancer families described evidence for a prostate cancer predisposition gene termed HPCX, which maps to Xq27-28. To confirm the potential contribution of this locus to prostate cancer susceptibility in an independent set of families, we studied 153 unrelated prostate cancer families who are participants in the University of Michigan Prostate Cancer Genetics Project. Families selected for this analysis have at least two living members affected with prostate cancer that are related in a way that they could potentially share one copy of the X chromosome. DNA samples were genotyped using a panel of seven polymorphic markers spanning 30 cM and containing the HPCX candidate region. The resulting data was analyzed using both nonparametric (NPL) and parametric linkage methods. Analysis of all 153 families resulted in positive NPL Z-scores across the entire candidate interval (NPL Z-scores 0.23-1.06 with corresponding one-sided p-values 0.41 and 0.15, respectively). The eleven African American families had negative NPL Z-scores across the same 30 cM interval. Analysis of the 140 Caucasian families produced a maximal NPL Z-score of 1.20 with a corresponding one-sided p-value of 0.12 at marker DXS1113. The subset of families with no evidence of male-to-male disease transmission and with early-onset prostate cancer (average age within a family < or = 65 years) contributed disproportionately to the evidence for linkage in the entire dataset [maximum NPL Z-score 1.51 (p-value 0.07) at DXS1108]. In conclusion, this study of 153 families, each with two or more living members with prostate cancer, provides some additional support for the existence of a prostate cancer susceptibility gene at Xq27-28.

Program Nr: 653 from the 1999 ASHG Annual Meeting

The LOXL2 gene, a putative tumor suppressor, maps to the minimally deleted region at 8p21 in lung, colon, prostate and breast cancer, and shows high frequency of allelic loss in colon and esophageal tumors. *K. Csiszar[1], S.F.T. Fong[1], C. Jourdan-Le Saux[1], L. Asuncion[1], E. Dietzch[2], M.I. Parker[2]*. 1) Pacific Biomedical Research Center, University of Hawaii, Honolulu, HI; 2) Dept of Medical Biochemistry, UCT Medical School, Cape Town, South Africa.

We have mapped LOXL2, a new member of the lysyl oxidase (LOX) gene family, to chromosome 8p21, the minimally deleted region in the four most common cancers, carcinomas of the lung, colon, prostate and breast. Although several genes have been localized to this locus, no tumor suppressors with demonstrated loss of function have been identified. LOXL2, a putative new tumor suppressor, has been shown to play a role in cell adhesion. Loss of this function, through frequent deletions and allelic losses of the LOXL2 gene, may contribute to the development of an invasive tumor phenotype in these malignancies. To confirm this hypothesis, we have localized the LOXL2 protein in normal colon and in colonic adenocarcinoma, examined the expression pattern of the LOXL2 gene in several tumor cell lines and assessed the deletional status of the LOXL2 gene using a new intragenic microsatellite in a series of colon and esophageal carcinomas. Immunohistochemical analysis localized the LOXL2 protein in the submucosa in normal colon but in the mucosa of a differentiated adenocarcinoma. Northern analysis of prostate and colon cancer cell lines and osteosarcoma cells indicated reduced mRNA levels in ras-transformed osteosarcoma (AD15) and in prostate cancer cells (DU145) and complete loss of expression of the LOXL2 gene in all colon cancer cell lines (HCT-116, HCT-15, DLD-1). Allelic changes of the LOXL2 gene were observed in 50% of 64 sporadic colon carcinomas, 16% of which were microsatellite instability (MI). In 68 esophageal tumor DNA samples, 36% demonstrated allelic changes with 7% MI. In both these cancers, males had more loss of heterozygosity (LOH) while females had more MI, indicating a gender-specific mutational mechanism. These results strongly suggest that the LOXL2 gene is affected by high frequency of deletions and may have an important role in the pathogenesis of colon and esophageal carcinomas.

Program Nr: 654 from the 1999 ASHG Annual Meeting

Combined use of microsatellite instability, immunohistochemistry and promoter hypermethylation to prescreen HNPCC mutations. *K.S. DAHAN[1], C. SEMPOUX[2], A. KARTHEUSER[3], M. SMAERS[1], P. CAMBY[2], R. DETRY[3], Ch. VERELLEN-DUMOULIN[1], J. RAHIER[2]*. 1) Genetics; 2) Pathology; 3) Surgery, U.C.L. Saint-Luc, BRUSSELS, BELGIUM.

Many studies demonstrated microsatellite instability (MSI) in tumors from hereditary non polyposis colorectal cancer (HNPCC) patients fitting the Amsterdam criteria. HNPCC is often associated with a germline mutation in 1 of the 2 major genes *hMLH1* and *hMSH2* responsible for repairing mismatches (MMR). However, MSI could be found in sporadic colorectal cancer (CRC) correlated to a MMR gene mutation in patients not fulfilling these criteria. To determine biological selection for these HNPCC, 92 consecutive surgical unselected CRC were studied. To evaluate the MSI, DNA from tumor and normal tissue was used for at least 4 (CA)n and 1 poly-A repeats. Three groups were defined: with <30% of markers showing instability (MSI-L, n=3, 3%), with >30% (MSI-H, n=16, 17%) and those without instability (MSS, n=73, 79%). Immunohistochemistry was performed on the same tumors with antibodies against hMLH1, hMSH2 and P53. No MSS nor MSI-L tumor had altered expression of either *hMLH1* or *hMSH2*. MSI-H cases demonstrated absence of staining for these proteins in 12 out 16(75%). Absence of hMLH1 occured in 10 tumors(83%). P53 expression was mainly focal in the MSI tumors (15 of 19, 79%) whereas it was diffuse in 3. Out of 73 MSS tumors, 56 (77%) had diffuse P53 activation whereas focal P53 expression was observed in 13 (no detection in 4).In 8 MSI-H CRC without hMLH1 expression, a *Hpa*II restriction enzyme assay was performed to look for promoter hypermethylation. The *hMLH1/calcitonin* ratio demonstrated hypermethylation of the *hMLH1* promoter in 1 of them. **Conclusion:** Out of 16 MSI-H tumors, normal expression of hMLH1 and hMSH2 is present in 4 whereas in 12, altered hMLH1 expression is responsible for CRC independent of P53 involvment. Lack of protein without promoter hypermethylation suggest either a germline or a somatic *hMLH1* mutation in 7 MSI-H tumors. The challenge to detect HNPCC from unselected CRC could be taken up by simultaneous analysis of MSI with protein expression and methylation status of the hMLH1 promoter.

Program Nr: 655 from the 1999 ASHG Annual Meeting

P53 genotyping is not an effective concept for molecular testing head and neck cancer. *R. Dahse[1], W. Fiedler[1], F. von Eggeling[1], S. Koscielny[2], E. Beleites[2], G. Ernst[1], U. Claussen[1].* 1) Institute of Human Genetics, Jena, Germany; 2) Clinic of ENT Diseases, Jena, Germany.

P53 mutations are currently recognized as the most common genetic alteration in human tumors. The purpose of our study was to prove the significance and reliability of p53 genotyping in head and neck cancer as a possible marker permitting the prediction of tumor behavior and clinical outcome. P53 genotyping in our study refers to highly sensitive molecular screening in order to detect structural alterations in the nucleic acid sequence of the gene. Exons 2-11 and adjacent intronic regions were screened for mutations by direct genomic sequencing or by bi-directional dideoxyfingerprinting in 66 primary tumors of the larynx, pharynx and oral cavity. Alterations in the hot spot region' of the p53 gene were detected in 36% (24 of 66) of the analyzed tumors, no mutation was found in our cohort outside exons 5-8. The frequency of p53 mutation did not correlate with the tumor stage or tumor site. The recurrence rate in patients with p53 alterations was not significantly higher compared to patients without a p53 mutation in their primary tumors. Summarizing the results of our study we found only limited reliability of p53 genotyping as an effective concept for prognostic differentiation in head and neck cancer. Molecular tests for tumor prognostication focusing on single parameters of cell cycle control seem to be insufficient owing to the complex interaction between many involved genes and proteins. Therefore, future efforts in searching for reliable prognostic markers should be focused on multiplex analysis systems for mutation detection or differences in gene expression patterns.

Program Nr: 656 from the 1999 ASHG Annual Meeting

Comparative genomic hybridization (CGH) in inherited and sporadic ovarian tumors in Israel. *M. Daniely[1], Y. Patael[2], W. Gottlieb[3], A. Aviram[1], A. Kruglikova[2], G. Ben-Baruch[3], E. Friedman[2].* 1) Human Genetics, Sheba Medical Ctr, Tel-Hashomer, Israel; 2) The Susanne Levy Gertner Oncogenetics Unit, Sheba Medical Center, Tel-Hashomer, Israel; 3) The Oncologic Gynecology Unit, Sheba Medical Center, Tel-Hashomer, Israel.

To gain insight into the molecular mechanisms involved in sporadic and inherited ovarian cancer, we analyzed 23 ovarian tumors form Jewish Israeli ovarian cancer patients by CGH: 18 invasive epithelial tumors (5 from BRCA1 or BRCA2 germline mutation carriers), two primary peritoneal carcinomatosis, and one each of pseudomyxoma peritoneii, sertoli cell tumor, and a normal ovary from a BRCA1 mutation carrier. The most common abnormality was amplification of 8q22.1-ter (in 8 primary tumors), followed by 1q22-32.1, 3q, 10p and 12p amplifications, and 9q and 16q21-24 deletions (noted in 3-4 tumors each). There were no differences between sporadic and inherited tumors; the type of genetic changes in primary peritoneal tumors and primary epithelial ovarian cancers, though the mean number of alterations in the latter was 8/tumor and in the former 3.5/tumor; there was an amplification of chromosome 6 in 2/3 metastases, which was not present in the primary tumor; amplification of 3q and deletion of chromosome 22 were restricted to women in whom the disease was diagnosed after the age of 55 years. Lastly, there were no alterations in the normal ovary, the pseudomyxoma peritoneii, and in metastases from a mucinus adenocarcinoma and its metastases. We conclude that a. the molecular mechanisms involved in ovarian cancer tumorigenesis in inherited and sporadic cases are closely related. b. multiple changes are involved in epithelial ovarian cancer c. the same mechanisms are operative in primary peritoneal carcinomatosis and d. pseudomyxoma peritoneii has a distinct molecular pathway for its development.

Program Nr: 657 from the 1999 ASHG Annual Meeting

Identification of proteins that interact with nibrin, the protein mutated in Nijmegen breakage syndrome. *A. Desai-Mehta[1,2], P. Concannon[1,2]*. 1) Molecular Genetics, Virginia Mason Research Center, Seattle, WA; 2) University of Washington School of Medicine, Seattle, WA.

Nijmegen breakage syndrome (NBS), an autosomal recessive disorder, is characterized by microcephaly, growth retardation, chromosomal instability, radiation sensitivity and a high incidence of malignancies, particularly of lymphoid origin. The NBS1 gene, encoding the protein nibrin, has recently been cloned and characterized in our laboratory. In normal fibroblasts, nibrin is localized to the nucleus and homogeneously distributed. Upon exposure of cells to ionizing radiation, nibrin forms distinct nuclear foci. The proteins, Mre11 and Rad50, which participate in DNA double-strand break repair, complex with nibrin in these nuclear foci and immunoprecipitation studies suggest the presence of additional, unidentified proteins. NBS cells which lack nibrin do not form foci upon irradiation, suggesting a role of nibrin in relocalizing this protein complex. The yeast two hybrid system was used to identify additional proteins that form part of these foci and interact with nibrin. Approximately two million clones from a human activated lymphocyte library were screened. After eliminating potential false positives, seven known genes were identified as well as several anonymous ESTs and genomic sequences. Given the defects in cell cycle checkpoint control in NBS cells, the identification of the G0/G1 transition gene GOS3 was of particular interest, as was the human Polycomb homolog hPc2 which is known to form nuclear foci, similar to nibrin. Two proteins, IL-2Rb and linker for T cell activation (LAT) involved in T cell proliferation, which is also defective in NBS cells, were identified although these proteins are localized to the cytoplasm. However, fluorescence microscopy revealed that, in contrast to fibroblasts, nibrin does not display nuclear localization in B and T cells thus raising the possibility of cytoplasmic interactions with IL-2Rb or LAT. All of these candidate interacting partners for nibrin are currently being tested for co-localization in cells and focus formation following irradiation.

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Program Nr: 658 from the 1999 ASHG Annual Meeting

Frequent splicing mutation of the ATM gene in breast cancer. *T. Dork[1], M. Nicke[1], K. Klopper[1], R. Bendix[1], M. Stuhrmann[1], M. Bremer[2], D. Rades[2], J.H. Karstens[2].* 1) Institute of Human Genetics; 2) Department of Radiation Oncology, Medical School Hannover, Germany.

The ATM gene that is mutated in ataxia-telangiectasia (A-T) encodes a large, predominantly nuclear protein kinase with an essential role in the repair of radiation-induced DNA damage. Obligate heterozygotes within A-T families have been shown to exhibit increased cellular radiosensitivity and to be at an increased risk to develop cancer, in particular breast carcinoma. We have searched for a frequent ATM gene mutation in German A-T families and in a population-based cohort of German breast cancer patients which should be useful in further epidemiological studies. Homozygosity for a new splicing mutation, 1066-6T®G in intron 10, was identified in 1 out of 30 A-T patients. This polypyrimidine tract substitution results in the loss of exon 11 in ATM mRNA from this patient which causes a frameshift. Heterozygosity for the same mutation was observed in 7 out of 870 consecutive breast cancer cases (0.8 %) and in 3 out of 450 controls from the general Lower Saxonian population (0.6 %). Transcript analyses in primary lymphocytes obtained from three of the heterozygous breast cancer patients confirmed exon skipping in about half of their ATM mRNA in each case. In conclusion, we have identified a new ATM gene mutation that is common in A-T and breast cancer patients and is frequent in the German and possibly other Central European populations. This finding enables a rapid molecular testing of A-T heterozygosity which will be useful for prospective studies of radiosensitivity and for large-scale association studies to investigate the relative risks of cancer in heterozygous carriers.

Program Nr: 659 from the 1999 ASHG Annual Meeting

Inherited risk of paraganglioma tumors. *C.M. Drovdlic[1], B.E. Baysal[2], B.J. Devlin[2], J. Peters[1,3], E. Myers[3], W.S. Rubinstein[1,3]*. 1) Univ. of Pittsburgh Graduate School of Public Health; 2) Dept. of Psychiatry, Univ. of Pittsburgh Medical Center; 3) Univ. of Pittsburgh Cancer Institute: Pittsburgh, PA.

Paraganglioma (glomus) tumors (PGLs), are mostly benign tumors of the head and neck which affect approximately 1 in 30,000 individuals. These growths can occur sporadically, but reportedly 7-50% are hereditary in nature. A major gene responsible for these tumors, *PGL1*, has been located on chromosome 11q23, but has not yet been identified. This gene is inherited in an autosomal dominant fashion, but exhibits genetic imprinting through maternal lineages such that children of carrier mothers are rarely, if ever, affected. This often makes heritable cases difficult to identify as the tumor can "hide" through several generations of maternal transmission. We are interested in determining the proportion of these tumors that are hereditary, identifying any other cancers or tumors associated with PGL, and assessing the PGL knowledge and attitudes of affected individuals. We will begin characterizing hereditary and sporadic tumors through acquisition of detailed family/medical history. We will identify patients with hereditary PGL by positive family history or multifocality. Cases in which the hereditary status is ambiguous will be analyzed molecularly by identifying subjects that carry a common PGL-1 disease marker. Through a probability model we will then determine the proportion of hereditary versus sporadic PGL in our subject population. Subjects will answer questionnaires to determine how well informed they feel about PGL, and how recurrence risk perception differs based on whether an affected individual believes he/she has a hereditary PGL. Attitudes towards possible future gene testing for affected individuals and their family members will also be assessed. The ability to identify hereditary PGL and any associated cancers or tumors serves to identify other family members at risk, which has important genetic counseling implications. The overall goal of this project is to add to the body of knowledge concerning these rare tumors in order to enable the refinement of genetic counseling and medical management for patients and families suffering from such tumors.

Program Nr: 660 from the 1999 ASHG Annual Meeting

Two polymorphisms in the VHL-associated gene *CUL2* are over-represented in pheochromocytoma patients without somatic *CUL2* mutations. *E.M. Duerr[1,2], O. Gimm[1], J.B. Kum[1,2], S.C. Clifford[3], S.P. Toledo[4], E.R. Maher[3], P.L. Dahia[1,2], C. Eng[1]*. 1) Human Cancer Genetics Program, Ohio State University, Columbus, OH; 2) Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA; 3) Department of Pediatrics and Child Health, University of Birmingham, Birmingham, UK; 4) Endocrine Genetics Unit, University of São Paulo, São Paulo, Brazil.

Most pheochromocytomas occur as sporadic tumors; about 10% have a hereditary background. These usually benign hereditary tumors are most frequently associated with multiple endocrine neoplasia type 2 (MEN 2A and 2B) and von Hippel-Lindau disease (VHL). While these two major hereditary forms of pheochromocytomas have been associated with germline mutations of the *RET* and *VHL* genes, respectively, the molecular pathogenesis of sporadic pheochromocytomas is largely unknown. Cul2 has been recently identified as a putative tumor suppressor and interacts with pVHL. We analyzed a series of 26 distinct pheochromocytomas for mutations in the whole coding region of this gene to determine whether *CUL2* plays a role in the pathogenesis of sporadic pheochromocytoma. Except for one sporadic tumor which had a hemizygous gene deletion, we did not find any somatic mutations in *CUL2*. However, we found three novel polymorphisms in the gene. One of these variants, IVS5-6C/T as well as another previously described one, c.2057G/A, were found to be over-represented amongst the pheochromocytoma patients when compared to a race-matched control group. 43 of 52 (83%) pheochromocytoma chromosomes tested carried the IVS5-6C/T variant C compared to 28 of 52 (54%) control chromosomes ($p<0.005$). Further, 34 of 48 (71%) pheochromocytoma chromosomes tested carried the c.2057G/A variant G compared to 22 of 48 (46%) control chromosomes ($p<0.01$). While our findings suggest that *CUL2* does not play a major role in the pathogenesis of pheochromocytomas, it remains unclear whether epigenetic mechanisms are involved in its inactivation in VHL-associated tumors. Of interest, the potential role of over-represented sequence variants acting as low penetrance susceptibility alleles for pheochromocytoma development requires further investigation.

Program Nr: 661 from the 1999 ASHG Annual Meeting

Reproducibility of a fluorescent-based test for three *BRCA1*/*BRCA2* mutations in blood and paraffin tissue samples. *S.M. Ebbers[1], G. Hirsh-Yechezkel[2], A. Chetrit[2], B. Modan[2], J.P. Struewing[1]*. 1) Laboratory of Population Genetics, NCI, Bethesda, MD; 2) Department of Clinical Epidemiology, Chaim Sheba Medical Center, Tel Hashomer, Israel.

The 185delAG and 5382insC mutations in *BRCA1* and the 6174delT mutation in *BRCA2* are common founder mutations among Jewish breast and ovarian cancer cases. We examined the reproducibility of a multiplex PCR-based assay in two different sample types (blood and paraffin slides) from the same person. Subjects were 40 women with ovarian cancer, 11 of whom carried one of the three mutations, drawn from a larger case-control study. For each subject, DNA from both a blood sample and a paraffin section on a slide were tested blindly. Paraffin samples were scraped off the slides, digested with a simple digestion buffer, and used directly in the PCR reaction. The assay consists of a multiplex PCR reaction for exons 2 and 20 in *BRCA1* and a portion of exon 11 in *BRCA2*. The forward primer pair of each set was labeled with a fluor such that the mutations, all small insertion or deletions, could be detected as length polymorphisms using an ABI 310 sequencer and Genescan analysis. After correcting data entry errors, the results of the assay were identical for blood and paraffin samples for 39 of the 40 subjects. Microsatellite analysis of the discrepant samples suggests that they were not derived from the same individual. Our results suggest that when testing for specific founder mutations in epidemiologic studies of *BRCA1/2*, paraffin-derived DNA may be an acceptable material for analysis.

Program Nr: 662 from the 1999 ASHG Annual Meeting

_BRCA_1, _BRCA_2 and Pedigree Genetic Analysis to Determine Genetic Risk in the UK Royal Marsden Hospital Tamoxifen Prevention Trial. *R.A. Eeles[1,2], T.P. Powles[2], S. Ashley[2], D.F. Easton[3], L. Assersohn[2], N. Sodha[2], M. Dowsett[1], B. Gusterson[1], A. Tidy[2], G. Mitchell[1,2], Z. Kote-Jarai[1]*. 1) Institute of Cancer Research, Sutton, Surrey, SM2 5NG, UK; 2) Royal Marsden NHS Trust, Sutton, Surrey, SM2 5PT, UK; 3) CRC Genetic Epidemiology Unit, Strangeways Research Laboratories, Cambridge, CN1 4RN, UK.

In the Royal Marsden Hospital tamoxifen prevention study, 2500 women at increased risk of developing breast cancer because of a family history of the disease were randomised to receive tamoxifen 20 mg daily or placebo for 8 years. 70 women developed primary breast cancer; 36 whilst on placebo, 34 on tamoxifen. Family history out to at least 2nd degree relatives was taken from all women in the study. DNA from peripheral blood from 67 of the 70 women was analysed for coding mutations in the *BRCA*1 and *BRCA*2 genes by CSGE analysis of the entire coding region of both genes. 7 mutations were found, 2 in *BRCA*1 and 5 in *BRCA*2, 4 would be expected to be pathogenic as these were nonsense/frameshifts. 3 were rare variants which were not present in 100 normal controls. The posterior probability of carrying a breast cancer predisposition gene in the individuals who developed breast cancer was assessed using the Cyrillic genetic risk package, based on the Claus model (Claus *et al.*, 1991*). 26 women had <50% posterior probability of harbouring a breast cancer predisposition gene and 44 had a ³50% chance of having a breast cancer predisposition gene. In the former group of 26 women, 8 had been taking tamoxifen and 18 placebo. In the group of women with ³50% probability of having a breast cancer gene, 26 had been taking tamoxifen and 18 placebo. The differences between the numbers of women taking tamoxifen who subsequently developed cancer in the two groups divided by <50% or ³50% genetic risk was significant at (p=0.04). These preliminary data suggest that tamoxifen prevention may be more effective in women with a <50% chance of harbouring a breast cancer predisposition gene. A meta analysis of the interaction of genetic status with tamoxifen chemoprevention effectiveness should be conducted to test this hypothesis.
*Claus et al. Am J Epidemiol, 48, 1991.

Program Nr: 663 from the 1999 ASHG Annual Meeting

Alterations of 1q and 16q and der (1;16) detected by FISH and CGH in breast lobular carcinoma in situ. *J.E. Etzell, S. DeVries, K. Chew, B.M. Ljung, F.M. Waldman.* Cancer Center, UCSF, San Francisco, CA.

Frequent gain of chromosome arm 16q (63%) and loss of 1q (79%) have been identified in infiltrating lobular carcinoma (ILC). Lobular carcinoma in situ (LCIS) may represent both a precursor to infiltrating carcinoma and a marker of an overall field defect. This study was undertaken to identify genomic alterations in LCIS and to investigate the role of formation of a der (1;16) (q10;p10) chromosome in the infiltrating and in situ lesions. Comparative genomic hybridization (CGH) was used to identify chromosomal alterations in 17 cases of pure LCIS (no evidence of invasive carcinoma) using DNA microdissected from archival sections following DOP-PCR amplification. The mean number of alterations was 1.9 per tumor for these in situ lesions, compared to 5.8 per tumor for invasive lobular tumors. Loss involving 16q was the most frequent alteration in LCIS (88%). Gain of 1q was present in 41% of cases, and was associated with 16q loss. Presence of a derivative (1;16) chromosome was determined by fluorescence in situ hybridization (FISH) using satellite probes for chromosome 1 (pUC1.77) and chromosome 16 (pHUR195 and pSE16-2). Co-localization of the chromosome 1 and 16 signals was used to define the presence of the derivative chromosome (counts greater than the control mean+2SD were considered positive). The infiltrating and in situ cancers both showed a high frequency of classical satellite co-localization (60% and 64%, respectively). Although weak signal intensity prevented scoring of co-localization using the pSE16-2 alpha satellite probe in the archival LCIS sections, this probe showed 95% co-localization with pUC1.77 in the invasive tumors (fresh imprint preparations were used). The higher frequency of pSE16-2 is consistent with its centromeric location relative to pHUR195. These data suggest that formation of a derivative (1;16) chromosome occurs in almost all infiltrating and in situ lobular carcinomas. E-cadherin, a tumor suppressor gene located on 16q, is abnormally expressed in most LCIS and ILC. We propose that formation of the der (1;16) is a mechanism leading to loss of 16q, which is then selected for during tumor development.

Program Nr: 664 from the 1999 ASHG Annual Meeting

Candidate genes causing familial oesophageal cancer associated with the skin condition tylosis (focal NEPPK).

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Focal non-epidermolytic palmoplantar keratoderma (NEPPK), or tylosis, is an autosomal, dominantly inherited disorder of the skin that manifests as focal thickening of the palmar and plantar surfaces. In three families, the skin disorder co-segregates with oesophageal cancer and oral lesions. The tylosis oesophageal cancer (TOC) gene locus has been mapped to 17q25, a region that may also be implicated in the development of sporadic squamous cell oesophageal carcinoma, Barrett's adenocarcinoma of the oesophagus, and breast cancer. We now show that the integration of the genetic and physical maps of this region refines the location of the TOC locus and maps a number of candidate genes and ESTs. Several structural candidates such as envoplakin (EVPL)and integrin b4 (ITGB4) have been excluded as the TOC gene on this basis. However, one other candidate gene and 2 ESTs have been mapped within the TOC minimal region. In addition, analysis of sequence data generated from one clone spanning approximately one third of the minimal region has identified further promising gene fragments. Investigation of tissue expression patterns and isolation of full length cDNA sequences will allow further chracterisation of these genes and lead to mutation analysis of patient samples. It now appears that we are close to isolating the gene responsible for this familial form of squamous cell oesophageal carcinoma that may also play a role in sporadically occurring disease.

Program Nr: 665 from the 1999 ASHG Annual Meeting

Jumping translocations in leukemia and lymphoma. *Y. Fan[1,2], K. Rizkalla[2], B.F. William[3], C.J. Engel[4].* 1) Cytogenetics Lab, London Health Sciences Ctr, London, ON, Canada; 2) Department of Pathology, The University of Western Ontario, Canada; 3) Blood Transfusion and Hematology Lab, St. Joseph's Health Ctr, London, ON, Canada; 4) Department of Surgery, London Health Sciences Ctr, London, ON, Canada.

Jumping translocation (JT) is a rare cytogenetic aberration in leukemia and lymphoma, and its etiologic mechanisms are not clearly known. We report two cases with jumping translocations. One had follicular lymphoma and JTs of 1q onto the telomeric regions of 5p, 9p and 15q in three cell lines, co-existing with the specific translocation t(14;18)(q32;q21). The second case had acute myeloid leukemia and JTs of 11q, as the sole aberration, onto multiple derivative chromosomes in each of the abnormal cells. A total of 17 telomeric regions were seen as the recipients of 11q in this case, and 9q was always involved as one of the recipients in all abnormal cells. FISH confirmed the identification of 11q material in the derivative chromosomes. While 1q has been the most common donor of acquired JTs, this is the first report on JTs of 11q. Different from all previously reported JTs which involved a single derivative chromosome in each cell line and led to a mosaic trisomy, multiple derivatives in most of the abnormal cells in this case had led to a tetrasomy, or a pentasomy of 11q. The pattern of chromosome involvement as the recipients of 11q appears to show a continuing evolutionary process of jumping, stabilization and spreading of the donor material onto other chromosomes. Cases with acquired and constitutional JTs in the literature were reviewed. Somatic recombinations between the interstitial telomeric or subtelomeric sequences of a derivative chromosome and the telomeric sequences of normal chromosomes are believed to be the underlying mechanism of JTs and their clonal evolution. Recombinations between the telomeric sequences may frequently occur in human malignant cells, but are not noticeable until a telomeric region has been involved in a rearrangement and has served as the vehicle for a jumping translocation.

Program Nr: 666 from the 1999 ASHG Annual Meeting

Both Homologues Of FRA3B Are Involved In Translocation In An Esophageal Adenocarcinoma. *J.M. Fang[1], M.F. Arlt[1], D.G. Beer[2], T.W. Glover[1].* 1) Department of Pediatrics & Human Genetics, University of Michigan, Ann Arbor, MI; 2) Department of Surgery, University of Michigan, Ann Arbor, MI.

Common fragile sites are loci that are especially sensitive to forming gaps or breaks on metaphase chromosomes when DNA synthesis is perturbed. They have also been shown to be hot spots for translocations, deletions and SCEs in vitro. Because of their high instability and their co-localization with translocation breakpoints, common fragile sites have been suggested to play a mechanistic role in chromosome breakage and rearrangement in cancer cells in vivo. Direct tests of this hypothesis are now possible with the cloning of common fragile site loci. FRA3B, the most frequently seen common fragile site, spans the FHIT gene, which shows deletions and aberrant transcripts in many different type of cancers. In addition to deletions within the FHIT gene, FRA3B may also lead a high rate of chromosome translocations that could influence genes in addition to FHIT. Two translocations in the FHIT gene have previously been reported. Here we report translocations in both homologues of chromosome 3 in a primary esophageal adenocarcinoma cell line. These cells show no FHIT transcript by RT-PCR. The karyotype revealed translocations of both homologues of chromosome 3: t(3;16) and t(3;4). The breakpoints of both translocations were narrowed to the FHIT/FRA3B region by FISH. Using 3' RACE, a chimeric transcript resulting from the t(3;16) was identified which contains exons 1, 2, and 4 of the FHIT gene and an extra ~400 bp of sequence which maps to chromosome 16p13.3. We are currently testing for other chimeric transcripts produced by the translocations in this tumor and for function of the transcripts. These data suggest that FRA3B, or other common fragile sites, can be "hot spots" for chromosome translocation and for creation of chimeric transcripts which may play a role in tumor development.

Program Nr: 667 from the 1999 ASHG Annual Meeting

Tetranucleotide repeat sequences are more stable than dinucleotide repeats in cultured mammalian cells. *R.A. Farber[1], J.S. Lee[1,2], J.L. Genova[1], M.G. Hanford[1]*. 1) Dept. of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC; 2) Chosun University, Republic of Korea.

There have been conflicting reports regarding the relative stabilities of microsatellites with repeat units of different lengths, based on various types of studies. We have made a direct comparison of the rates of insertion and deletion mutations for a dinucleotide-repeat sequence [$(CA)_{17}$] and a tetranucleotide-repeat sequence [$(AAGA)_{17}$] in immortalized, nontumorigenic, mismatch-repair-proficient mouse CAK cells and in the H6 clone of the mismatch-repair deficient (*hMLH1*$^-$) colorectal cancer cell line HCT116. A plasmid vector that contained either of these sequences near the 5' end of a neomycin-resistance (*neo*) gene, such that the gene was translated out of frame, was transfected into the cells by electroporation, and transfected clones were selected for resistance to hygromycin. (The plasmid became integrated into the genome of the recipient cells.) Fluctuation analysis was carried out to measure the rates of mutation to resistance to the neomycin analogue G418. Mutation rates of the tetranucleotide repeat were approximately 10-fold lower than those of the dinucleotide repeat in the mismatch-repair-proficient cells and about 5-fold lower in the mismatch-repair-deficient cells. In addition, up to 52 independent H6 subclones were assayed by PCR for instability of several different endogenous uninterrupted tetranucleotide and dinucleotide repeats. The tetranucleotide repeats were found to be 3- to 15-fold more stable than the dinucleotide repeats (mean difference = 5-fold). The results of these direct analyses demonstrate that tetranucleotide repeats have considerably greater stability than dinucleotide repeats in two different types of cultured somatic cells.

Program Nr: 668 from the 1999 ASHG Annual Meeting

Family history information: developing a cancer risk assessment strategy. *C.D. Farrell, M.J. Rosenblatt, M. Skipper.* Roswell Park Cancer Inst, Buffalo, NY.

The ability to determine cancer risk status is important for both medical management and for identification of patients as candidates for participation in cancer genetic research. The best method for assessment of inherited risk is family history.

A comparative study of 3 different sources of family history information for a given patient was undertaken to determine the extent and type of information obtained, existence of discordances and omissions, and the ability to use this information to categorize cancer risk. Medical records of 192 randomly selected patients were reviewed for preselected elements relevant to risk classification. Of these 192 patients, 125 agreed to provide their information by telephone interview, and 83 had previously completed a voluntary questionnaire. In order to assign cancer risk, criteria were developed based upon established criteria for known cancer genetic syndromes and integrating phenotypic characteristics documented in association with increased risk for cancer associated genes. The classifications were designated as: high, moderate, general population, or indeterminate.

Preliminary results indicate that family history information was obtained on all patients; however, some information necessary for risk categorization was deficient in 33.9% of medical records. The family history information generally was found in 1-3 places, but this was not consistent across all records. There were 45 different locations noted, supporting the necessity for one designated location that would allow for access and updating. Telephone interview resulted in the ability to categorize 84.6% of those designated indeterminate by medical records. Concordance of risk categorization was 55.2% between the medical record and the telephone interview. There was greater concordance between the telephone interview and the questionnaire, but there were fewer numbers in this comparison. Further results of this study will be discussed, as will the risk categorization criteria and elements designated as critical for family history risk assessment.

Program Nr: 669 from the 1999 ASHG Annual Meeting

Genomic Differences within cystic and squamous components of an ovarian teratoma when grown in Primaria Plastic and Normal Tissue Culture Plastic with RPMI or LHC-9 media. *S.A. Faruqi, R.B. Deger, H. Javid, J.S. Noumoff.* OB/GYN, Div Gynecologic Oncol, Crozer-Chester Medical Ctr, Upland, PA.

Benign cystic and malignant squamous components of an ovarian teratoma were grown simultaneously on Primaria, a positively charged dish (Beckton Dickinson,Franklin Lakes,N.J.), and normal tissue culture plastic (NCTP)using two different media, RPMI with serum and the serum free media LHC-9 (Biofluids, Rockville, MD). The cyst showed both diploid and polyploid cells in the Primaria with RPMI (32:7) and in the Primaria with LHC-9 (28:1) whereas no polyploid cells were seen in the NTCP with RPMI (27:0) or in the NTCP with LHC-9 (16:0).
The squamous component did show both diploids and polyploids in all cultures except Primaria with LHC-9, where dividing cells were absent in the majority of the cultures. Only one culture out of three showed two dividing cells, both of which were polyploids. Both cystic and the squamous components contained significant variations in the presence or absence of certain specific markers. The marker del(4)(q22.1)reported earlier in dermoid tumor was also present in this material as well and so was trisomy 12 which is generally present in most of the germ cell tumors. The most interesting abnormality found both in cystic and squamous components was del(22)(q12.1) but a translocation with chromosome 9 could not be detected. Deletion 22q was seen in the NTCP with either RPMI or LHC-9 but it was absent in Primaria. The chromosomal variation within and between these cultures have significant implications in the complete recovery of the tumor genome.

Program Nr: 670 from the 1999 ASHG Annual Meeting

Human papillomavirus (HPV) study of 482 pathological specimens by PCR-direct sequencing (PCR-DS) approach. *J.C. Feoli-Fonseca[1], L.L. Oligny[1], P. Brochu[1], P. Simard[1], W.V. Yotov[2].* 1) Pathology, Hosp. Ste-Justine, U. Montreal, Montreal, Quebec, Canada; 2) Pediatrics, Hosp. Ste-Justine, U. Montreal, Montreal, Quebec, Canada.

Human papillomaviruses (HPV) are etiological agents for cervical cancer and some other human diseases. Using our two-tier PCR-direct sequencing (PCR-DS) approach for HPV detection and typing, we tested 482 pathological specimens and found 354 of them (73.4%) HPV-positive. Most of the specimens (79%) were diagnosed as cervical intraepithelial neoplasia (CIN) grade I-III. Four most frequently found isolates accounted for 56 % of all sequences: HPV6, 16, 11 and 31 (24.2%, 20.5%, 6.6%, and 4.5%, respectively). Some unexpected results of our study are: strong relative increase of HPV types of unknown cervical cancer risk in co-infections; high, for a PCR-based method, number of individual viral isolates (sequences) from co-infections (20.7% of all isolates); higher incidence of high-risk HPV types 31 and 56 than HPV18; new data indicating that the HPV evolutionary patterns are quite complex. We placed for the first time some novel (identified by our study) and known human and animal papillomaviruses in a common clade, contradicting previous hypothesis that genetic diversity in HPV came late in evolution, after the divergence of the human species from its non-human predecessors. In the course of this study we created a new HPV database for quick search and identification of known and novel HPV types based on a short part of the consensus region determined by primers MY09/11 and GP5/GP6. To our knowledge, this is the largest sequencing-based single-laboratory study of HPV.

Program Nr: 671 from the 1999 ASHG Annual Meeting

Differential analysis of telomerase activity in microdissected breast cancer samples. *W. Fiedler[1], C. Mueller[1], U. Riese[1], R. Dahse[1], F. von Eggeling[1], H. Kosmehl[2], G. Ernst[1], U. Claussen[1]*. 1) Inst Human Genetics, Jena, Germany; 2) Inst Pathology, Jena, Germany.

A differentiated molecular analysis of tumor tissue samples is complicated in general by three aspects: (i) genetic heterogeneity of the tumor tissue itself, (ii) morphological heterogeneity within the samples and (iii) the a priori unknown ratio between tumor cells and non-tumorous cells within the sample. To overcome these problems in the analysis of telomerase activity in breast cancer we performed microscopic evaluation of each tumor sample and subsequent tissue microdissection of unstained cryostat sections under an inverted microscope with the help of a micromanipulator and extended glass needles. Thus, the specific assignment of telomerase activity to distinct tissue areas including small lesions and stroma cells was possible in 134 cases of breast cancer. In total, 108 of them (80 %) expressed telomerase. Corresponding non-tumorous tissue stroma free of infiltrating lymphocytes was telomerase negative in 41 samples tested. Only 3/10 ductal carcinoma in situ (DCIS) exhibited telomerase activity. Variations of the telomerase status were found in 34 of 73 (45 %) samples by analyzing several areas of one tumor. In 6 of 23 samples intraductal and invasive growing tumor areas analyzed in parallel, the finding of different levels of telomerase activity displayed tumor heterogeneity. Our results indicate that microdissection of histological sections enables investigations of pure populations of morphologically well defined cells. The direct correlation of the telomerase activity to specific histological findings provide a more differentiated view into cancerogenesis and tumor progression.

Program Nr: 672 from the 1999 ASHG Annual Meeting

BTAK overexpression is associated with aggressive bladder cancer. *G.C. Fraizer[1], H. Zhou[2], M. Diaz[1], C. Joy[1], I. Lee[1], C. Reznikoff[3], M. Liebert[1], H.B. Grossman[1], S. Sen[2].* 1) Urology,Univ. of Texas M.D.Anderson Cancer Ctr., Houston, TX; 2) Pathology and Lab Medicine, Univ. of Texas M.D.Anderson Cancer Ctr., Houston, TX; 3) Human Oncology, Univ. of Wisconsin.

Accumulation of extensive genetic abnormalities and chromosomal aneuploidy has been associated with high stage, aggressive bladder cancer. Specifically, amplification of chromosome 20q has previously been associated with invasive bladder cancer, but the gene(s) involved were unknown. We hypothesize that a candidate gene for invasive bladder cancer development may be BTAK, a centrosome associated serine/threonine kinase that has recently been identified and located at 20q13 in the amplicon that is often amplified in bladder tumors. This finding led us to ask whether BTAK may be overexpressed in bladder tumors. Using immunofluorescence staining, northern and western blot analyses, we present the first evidence that BTAK expression is higher in some bladder tumors and cell lines than in adjacent normal bladder tissue and normal urothelial cells. We also demonstrate that BTAK expression levels are correlated with tumor stage. BTAK expression is: 1) higher in invasive bladder tumors than non-invasive, 2) low in bladder tumor cell lines derived from low grade tumors and 3) higher in an E7 transformed urothelial cell line with 20q amplification than in an E6 transformed line which lacks 20q amplification. Our hypothesis is that high BTAK expression in bladder urothelium leads to aneuploidy and aggressive tumor development. While superficial bladder tumors commonly recur, most never progess to invasive and potentially lethal bladder cancer. Identifying biomarkers, such as BTAK, that may indicate which tumors are likely to progress can help guide appropriate therapy. Furthermore because it is likely that aneuploidy contributes to tumor progression, understanding mechanisms of aneuploidy will be important for the development of new treatments directed at biologically aggressive bladder cancer.

Program Nr: 673 from the 1999 ASHG Annual Meeting

Germ-line *NF2* mutation type, location, and phenotype in neurofibromatosis 2. *J.M. Friedman[1], R. Woods[2], H. Joe[2], D.G.R. Evans[3], A. Wallace[3], V.F. Mautner[5], L. Kluwe[4], D.M. Parry[6], G.A. Rouleau[7], M.E. Baser[8].* 1) Dept. of Medical Genetics, Univ. of British Columbia, Vancouver, Canada; 2) Dept. of Statistics, Univ. of British Columbia, Vancouver, Canada; 3) St. Mary's Hospital, Manchester, U.K; 4) University of Hamburg, Hamburg, Germany; 5) Klinikum Nord Ochsenzoll, Hamburg, Germany; 6) National Cancer Institute, Bethesda, U.S.A; 7) McGill University, Montreal, Canada; 8) Los Angeles, USA.

Germ-line neurofibromatosis 2 (*NF2*) mutation type is associated with phenotypic differences, but the effect of mutation location is unknown. We reviewed NF2 genotype-phenotype data from 101 patients in 47 families with splice-site mutations, and 198 patients in 161 families with nonsense or frameshift mutations. Patients were stratified by mutation type and proband status, then group comparisons were made in probands using the t-test or Fisher's exact test, with similar comparisons made in non-probands by means of a mixed effects model to allow for familial dependence. Probands with splice-site mutations had a higher age at onset of symptoms (mean $\pm$ SE: 23.9 $\pm$ 2.0 yrs versus 18.0 $\pm$ 1.0 yrs, p = .007) and a higher age at diagnosis (31.5 $\pm$ 2.5 yrs versus 23.9 $\pm$ 1.2 yrs, p =.004) than probands with nonsense or frameshift mutations. Non-probands with splice-site mutations had a higher age at onset of symptoms (23.2 $\pm$ 2.1 yrs versus 17.6 $\pm$ 1.5 yrs, p = .034), fewer intracranial meningiomas (0.5 $\pm$ 0.7 versus 1.9 $\pm$ 0.6, p = .027), and possibly a lower prevalence of spinal tumors (46.5% versus 66.7%, p = .078) than non-probands with nonsense or frameshift mutations. Mutation location (exons 1-13 versus 14-15) was not significantly associated with number of meningiomas, presence of spinal tumors, or age at diagnosis in either mutation group. However, the age at onset of symptoms was higher in non-probands with splice-site mutations affecting exons 14-15 compared to exons 1-13 (30.5 $\pm$ 3.7 yrs versus 20.6 $\pm$ 2.3 yrs, p = .034). No such association was seen among patients with nonsense or frameshift mutations. These results are consistent with previous NF2 genotype-phenotype correlation studies, and suggest that germ-line *NF2* alleles with splice-site mutations in 3' exons may produce a partially functional protein product.

Program Nr: 674 from the 1999 ASHG Annual Meeting

Functional homologies shared between ATM and TEL1p from *S.cerevisiae*. *E. Fritz[1], A.A. Friedl[2], F. Eckardt-Schupp[1], M.S. Meyn[3].* 1) GSF, Inst Radiation Biology, Neuherberg, Germany; 2) Dept of Radiobiology, University of Munich, Germany; 3) Hospital for Sick Children, Toronto, Canada.

Atm, the gene defective in the human genetic disorder ataxia-telangiectasia (A-T), is a member of a family of eukaryotic proteins that are involved in regulating cellular responses to DNA damage. The *S.cerevisiae* protein Tel1p is the closest known homolog of ATM, sharing 45% identity in the kinase domain and 21% in the rest of the protein. Tel1-deficient yeast cells express a similar phenotype to that of cultured A-T cells, including chromosome loss, hyperrecombination and telomere shortening. The high sequence homology and overlapping phenotypes of the two mutants prompted us to analyze functional properties shared between ATM and Tel1p in cross-complementation analyses. The complete *TEL1* coding sequence (8361 bp) was cloned into a mammalian expression vector. Upon stable transfection into A-T fibroblasts, expression of *TEL1* was confirmed by RT-PCR. Phenotypic analyses of *TEL1*-transfected A-T cells showed that i) *TEL1* reduces hyperrecombination, as measured in fluctuation analyses of spontaneous intrachromosomal recombination events; ii) *TEL1* partly complements ionizing radiation (IR)-induced apoptosis, but not reduced colony survival of A-T cells after IR; iii) *TEL1* extends the length of telomeres, as measured by analyzing the mean length of terminal restriction fragments; iv) *TEL1* had no effect on basic and IR-induced cell cycle progression as well as basic and IR-induced p53 protein amount and DNA binding activity. These data indicate that TEL1p can partially substitute for ATM in human cells and strongly suggest that TEL1p shares functional properties with ATM. In addition, our results demonstrate that the hyperrecombination phenotype of A-T cells can be uncoupled from cell cycle regulation. By complementing a specific subset of A-T defects, *TEL1*-transfected A-T cells may provide useful tools to decipher the multiple cellular pathways regulated by ATM. Analyses of protein expression and activity in TEL1p-complemented A-T cells should allow insights into how p53 and other proteins downstream of ATM regulate genetic recombination, telomere metabolism and radiosensitivity.

Program Nr: 675 from the 1999 ASHG Annual Meeting

Loss of heterozygosity in Renal Cell Carcinoma and correlation with clinicopathological data. *C. Gallou[1], D. Joly[2], A. Méjean[3], R. Grifone[1], M. Perennou[1], D. Droz[4], R. Bouvier[5], C. Junien[1], C. Béroud[1]*. 1) INSERM U 383, hôpital Necker-Enfants malades, PARIS Cedex 15, FRANCE; 2) Service de néphrologie, hôpital Necker-Enfants malades, PARIS Cedex 15, FRANCE; 3) Service d'urologie, hôpital Necker-Enfants malades, PARIS Cedex 15, FRANCE; 4) Laboratoire d'anatomie pathologique, hôpital Necker-Enfants malades, PARIS Cedex 15, FRANCE; 5) Laboratoire d'anatomie pathologique, hôpital Edouard Herriot, LYON, France.

Renal Cell Carcinoma (RCC) accounts for more than 90% of adult kidney cancer and is the eleventh most frequent cancer. Cytogenetic and molecular studies showed several recurrent abnormalities including alterations of chromosome 14 in 30% of cases. These data indicates that at least one gene is involved in renal tumorigenesis on this chromosome. A previous study in our laboratory, on 148 tumors, led to the following conclusions: a) there is a loss of 14q (LOH) in 28% of RCC; b) a significant correlation between the loss of 14q and the high grade or stage of the tumor is observed; c) no commonly deleted region can be defined. To confirm these results and identify a candidate region, we collected 100 new tumors and used 14 microsatellites markers (CA)n spreading along the long arm of chromosome 14 to look for LOH. Preliminary results show a partial or complete loss of 14q in 37% of cases. Two tumors have a partial loss of the chromosome and allowed the definition of a commonly deleted region localized between 14q21.3 and 14q23.3. The study of new regional markers and other tumors should help to refine this interval. We confirm the correlation between 14q LOH and the two main prognostic factors of RCC: tumors grades and stages. We observe 14q LOH in 39% of tumor of low stage (I-II) versus 60% for high stage (III-IV) and an increase of 33% to 62% for the tumors of grade 1 to 4. These data suggest that there is at least one tumor suppressor gene on chromosome 14 and that his alteration is critical for malignant phenotype. So, the loss of 14q could be considered as a new prognostic factor if we can demonstrate that it is independent from the tumors grades and stages. A survival study on patients studied in 1993 is in progress to validate this hypothesis.

Program Nr: 676 from the 1999 ASHG Annual Meeting

BRCA1 mutation analysis in at-risk African-American Families: results and implications. *L. Gayol[1], T. Scholl[2], H. Basterrechea[1], I. Pfeifer[1], J. Davies[1], E. Perera[1], S. Smith[3], J.F. Arena[1], L. Baumbach[1]*. 1) Pediatrics, Univ. of Miami Sch. of Med, Miami, FL; 2) Myriad Genetics Laboratories, Salt Lake City, UT; 3) Epidemology, Univ. of Miami Sch. of Med, Miami, FL.

The incidence of *BRCA1*germ-line mutations in at-risk individuals is controversial. In Caucasians, the detection of *BRCA1* mutations varies from 5-40%. Even more controversial is the incidence of *BRCA1* mutations and genetic variants in at-risk African-Americans (AA), which has been reported as ranging from very low to incidences equalling those in Caucasians.

We report our results of completed analyses of *BRCA1* in 20 AA families at-risk for breast/ovarian cancer. These families are subdivided into three categories: high-risk (HR); three affected 1st degree relatives; 10 families), moderate-risk (MR; two affected 1st degree relatives, 7 families) and undetermined risk (UR; single affected with medical information being updated, 3 families). We have screened the entire *BRCA1* coding sequence for mutations and other genetic variants using exon-specific PCR primers, followed by SSCP analysis, and DNA sequencing as described (AJHG,63:A325).

To date, we have identified only <u>one</u> deleterious mutation (3875 del GTCT), which occurred in a MR family. However, two different AA-specific intronic polymorphisms were detected in two HR unrelated families in which a *BRCA1* disease-causing mutation was not otherwise detected. To investigate a possible effect on *BRCA1* splicing, analysis of *BRCA1* mRNA was performed using DNA and RNA extracted from patients' peripheral blood. These results indicated that wild-type *BRCA1* mRNA was produced from variant-containing alleles, although alternatively spliced products, including deleted transcripts, were detectable at low levels. In addition, a novel missense mutation was detected in exon 19 (W1718C) in the second HR family only, and appears to co-segregate with breast cancer. In conclusion, our data support a low germ-line *BRCA1* mutation rate in AA patients, and suggest a possible role for AA-specific *BRCA1* variants in modulating breast cancer risk.

Program Nr: 677 from the 1999 ASHG Annual Meeting

Pathogenetic significance of constitutional missense mutations of the cancer-predisposing genes *MLH1* and *MSH2*. *M. Genuardi[1], S. Carrara[1], L. Della Puppa[2], O. Caluseriu[1], V. Rovella[1], M. Ponz de Leon[3], M. Pedroni[3], M. Anti[4], M. Boiocchi[2], A. Viel[2], G. Neri[1]*. 1) Institute of Medical Genetics, Catholic Univ Sch Medicine, Rome, Italy; 2) Experimental Oncology 1, Centro di Riferimento Oncologico, Aviano; 3) Department of Internal Medicine, Univ of Modena; 4) Institute of Internal Medicine and Geriatrics, Catholic Univ Sch Medicine, Rome, Italy.

Hereditary nonpolyposis colorectal cancer or Lynch syndrome (LS) is a cancer-predisposing condition determined by mutations in genes involved in the DNA mismatch repair (MMR) system. The majority of constitutional mutations detected in LS families affect the *MLH1* and *MSH2* genes by determining the production of truncated polypeptides. Missense mutations are also observed, but their pathogenetic significance has not been systematically ascertained so far. To determine the role played by *MLH1* and *MSH2* missense variants in cancer susceptibility, we have investigated the following genetic and biological characteristics associated with 6 *MLH1* and 4 *MSH2* missense changes identified in Italian LS families: 1) co-segregation with disease phenotype and/or *bona fide* pathogenetic mutations; 2) presence of the variant in healthy control subjects; 3) evolutionary conservation of the involved aminoacid and type of aminoacid change; and 4) presence/absence of microsatellite instability (MSI) in tumor DNA, which can be considered as a sort of *in vivo* biological assay aimed at determining the functional status of the MMR machinery. Overall, 9 variants did not fulfill $\geq$2 pathogenicity criteria. MSI was investigated in tumor samples from carriers of 9 different missense mutations. Only 3/9 tested variants were associated with MSI in tumor DNA. In addition, 4 variants were not present in affected pedigree members, and 5 variants were observed at low frequencies in the control population. Based upon these results, we conclude that most *MLH1* and *MSH2* missense changes investigated in this study are unlikely to act as major causative factors in colorectal cancer susceptibility and development.

Program Nr: 678 from the 1999 ASHG Annual Meeting

The generation of a sequence-ready contig encompassing the cervical tumor suppressor gene (TSG) on 6p23. *D.S. Gerhard[1], Z. Xu[1], L. Nguyen[1], Y. Li[1], A. Mungall[2], S. Beck[2], P. Huettner[1], J.S. Rader[1]*. 1) Depts of Genetics, Gynecology/Oncology, Pathology, Box 8232, Washington University School of Medicine, St Louis, MO; 2) Sanger Centre, Wellcome Trust Genome Campus, Hinxton, England.

Invasive cervical cancer (ICC) is the fifth most common malignancy in the world, with ~500,000 cases each year. Infection by the human papillomavirus (HVP) increases the risk of cervical cancer; 75-90% of tumors are virus-positive. However, HPV infection alone is not sufficient for the development of cervical cancer since up to 70% of women in some populations are infected, yet the malignancy develops in less than 8%. We have previously showed that a large number of cervical tumors have lost all or part of 6p, suggesting that a TSG is localized there. A study of the precursor lesions, cervical intraepithelial neoplasia, found that they too had deletions of 6p. Characterization of 107 tumors with 22 markers spanning about 70 cM of 6p and found that 57/107 (53%) lost at least 6p21-6p23; some deletions were larger. Fifty-two out of 57 tumors (91%) had a loss at 6p23. Deletions were found in tumors of all stages and histologic types. Seven tumors appear to have small interstitial deletions of 6p23 and they provide the boundaries of the candidate region. The smallest region of loss is flanked by D6S443/S429 and D6S1578, loci separated by ~1 cM. We obtained YACs containing markers from this location that had been previously identified by others. The clones were characterized with the polymorphic markers used in the tumor analysis as well as STSs and ESTs provisionally mapped to this region. We used SEGMAP to generate the size and estimate the distance between the markers. We then converted this contig into PACs and BACs. The conversion was done by: a) PCR screening of RPCI1, 3 and 11 libraries (2 PACs and 1 BAC) and b) search of sequence databases. We identified 82 clones spanning a region of ~ 1Mb, with an average depth of 8 clones per marker. All clones were assayed for their STS content. This contig is a valuable resource in the search for the cervical TSG.

Program Nr: 679 from the 1999 ASHG Annual Meeting

Different subcellular localization and cell cycle expression patterns of the Bloom syndrome and Werner syndrome helicases. *V. Gharibyan, H. Youssoufian.* Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Bloom syndrome (BS) and Werner syndrome (WS) are caused by deficiencies of a related class of DNA helicases. However, their phenotypes differ markedly. BS is characterized by growth retardation, immune deficiency, cancer susceptibility and excessive numbers of sister chromatid exchanges, while the major feature of WS is premature aging. To begin to understand the molecular basis for the phenotypical differences, we generated a novel polyclonal antibody to the human BS helicase, Blm, and compared the subcellular localization and expression of Blm to the WS helicase, Wrn. We detected the 170 kDa Blm antigen in wild type but not BS cells. Blm was localized to punctate nuclear structures. The level of Blm but not Wrn was 3.6 fold-higher in G1/S- compared to G0-synchronized fibroblasts. Blm-positive cells invariably expressed topoisomerase IIa, while topoisomerase IIb was expressed constitutively. Transfections of Blm deletion mutants demonstrated that the C-terminal domain mediates nuclear entry and the central helicase domain is necessary for producing the punctate pattern. By subcellular fractionation, Blm was found primarily in high-salt extracts of the nucleoplasm and the nuclear matrix and was enriched in G1/S- compared to G0-synchronized cells. There was no interaction between Blm and Wrn or topoisomerases IIa and IIb in extracts from fibroblasts. These results demonstrate that Blm is targeted to specific nuclear structures, and its expression is enhanced during cell growth. The known nucleolar localization of Wrn, its invariant expression during the cell cycle, and the lack of interaction between Blm and Wrn suggest distinct roles for Blm and Wrn in processes such as DNA repair or recombination.

Program Nr: 680 from the 1999 ASHG Annual Meeting

Expression profile of the adrenal gland from patients with primary bilateral adrenocortical diseases: Primary pigmented adrenocortical disease (PPNAD) (associated with Carney Complex) and massive macronodular adrenocortical disease (MMAD). *C. Giatzakis, S.E. Taymans, C.A. Stratakis.* UGEN, DEB, NICHD, NIH, Bethesda, MD.

The use of high density cDNA filter arrays (HDFA) to compare the expression of thousands of genes between normal and pathologic tissues, allows for the identification of multiple potential target genes involved in adrenocortical tumorigenesis. This study focuses on the expression profile of the adrenal gland from patients with PPNAD associated with Carney Complex (CNC-MIM160980) and MMAD. The adrenal gland of PPNAD is characterized by multiple, small, pigmented (black or brown), adrenocortical cortisol-producing nodules surrounded by internodular atrophy within normal-sized or small glands. Patients with PPNAD exhibit ACTH - independent hypercortisolism and mostly atypical forms of Cushing syndrome (CS). MMAD, unlike PPNAD, almost always leads to frank CS and the adrenal glands are massively enlarged. An HDFA containing approximately 4000 IMAGE cDNA clones was used to examine the expression profile of 3 PPNAD tumors from patients with CNC and one adrenal gland derived from a patient with MMAD. The expression profile of a normal adrenal gland was used as a control. Comparative expression analysis of the PPNAD adrenals versus the normal revealed 8 array elements that displayed altered density ratios of >2 fold in all three PPNAD specimens. All of those differentially expressed genes were repressed in the PPNAD. Three genes exhibited the greatest differences: a) Ubiquitin carrier protein E2-EPF, b) Ubiquitin conjugating enzyme G2, c) Tuberin. Analysis of the expression profile of the MMAD adrenal revealed a greater number of putative differentially expressed genes at a ratio of >2 (approx. 260 genes). We conclude that HDFA is an effective and reproducible way to study differential gene expression. We also conclude that abnormalities of the ubiquitin conjugating enzyme system may be important steps in the process of adrenocortical tumorigenesis in PPNAD. This finding may provide a molecular link between PPNAD/CNC and the chromosomal instability that is observed in this condition.

Program Nr: 681 from the 1999 ASHG Annual Meeting

Scoresheet for family physicians to evaluate family history of breast and ovarian cancer. *C.A. Gilpin, N.L. Carson, A.G.W. Hunter.* Dept. of Genetics, Children's Hospital of Eastern Ontario, Ottawa, ON, Canada.

Breast and ovarian cancers are relatively common conditions in the general population with 5-10% of cases being due to an inherited predisposition. The medical community and general population have become aware that genetic testing is available to look for BRCA1 and BRCA2 mutations, but what is not clear to these groups is who should be considered for the genetic counselling and possible subsequent testing. In Canada, BRCA1 and BRCA2 testing is available through research projects to defined high-risk families, at no monetary cost to the individual. Genetic counselling is also available through research programs and these programs have been overwhelmed by interest from the public. A scoresheet was developed for referring physicians to evaluate a family's genetic risk of familial breast or ovarian cancer. This simple tool identifies high-risk families who could be considered for genetic counselling. A more complex assessment is carried out in the Genetics clinic to determine if an individual meets testing criteria.

A comparison study of risk assessment methods was completed for 143 unaffected probands, where genetic testing of an affected sister was complete. Assessments using Claus tables and Bayesian analysis and probability calculations in BRCAPRO were compared to our scoresheet values. A cut-off value was assigned, above which a woman should be referred for genetic counselling and below which her risk of breast or ovarian cancer is not significantly increased. Our target was to ascertain women with double the lifetime risk for breast or ovarian cancer. We have shown that this scoresheet consistently identifies women whose calculated lifetime risk of developing breast cancer (100%) and ovarian cancer (77%) is double that of the general population. It identified 97% of women from families where a mutation was found. Those missed were from low-risk control families.

Program Nr: 682 from the 1999 ASHG Annual Meeting

Genotype-phenotype relationships in American melanoma-prone families with CDKN2A and CDK4 mutations.
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Two genes have been implicated in malignant melanoma (MM) pathogenesis. CDKN2A/p16 is a tumor suppressor gene with germline mutations detected in about 25% of MM families some of whom also have pancreatic cancer. CDK4 is an oncogene with germline mutations found in only 3 kindreds. We compared 104 MM cases from 17 p16 families and 12 MM cases from 2 CDK4 families. Recurrent p16 mutations were Val126Asp (n=3 families) and Gly101Trp (n=4). Five p16 families (29 MM cases), each with different mutations, Arg87Pro, Gly101Trp, Val126Asp, IVS2+1, 234del14, had pancreatic cancer. Both CDK4 families had the Arg24Cys mutation. We used nonparametric statistics to test for differences in median age at first MM diagnosis and numbers of MM tumors. The three recurrent mutations were haplotyped to assess whether the alterations occurred de novo or were founder mutations. Despite the hypothetical differences of action between the tumor suppressor p16 and dominant oncogene CDK4, median age at MM diagnosis (34.2 years, p=.70) and numbers of MM tumors (1.0, p=.73) were indistinguishable between MM cases from p16 versus CDK4 families. The major difference between the two groups was the association of pancreatic cancer in the p16 families. However, there were no significant differences in median age at diagnosis (p=.10) or tumor number (p=.58) between MM cases from families with pancreatic cancer (31.2 yrs; 1.0 tumors) versus families without pancreatic cancer (36.2 yrs; 1.0 tumors). Analysis of markers flanking the CDKN2A locus revealed common haplotypes for families with the Val126Asp and Gly101Trp mutations. These two founder mutations were observed in families with and without pancreatic cancer suggesting that other factors, genetic and/or environmental, may be involved in the development of pancreatic cancer. The CDK4 families, one of Irish and one of German descent, also shared a common haplotype at 6 markers flanking CDK4. Thus, the 3 recurrent missense mutations observed in these MM families- Val126Asp and Gly101Trp CDKN2A mutations and the Arg24Cys CDK4 mutation- each showed evidence for a common founder regardless of the mechanism of action of the respective gene.

Program Nr: 683 from the 1999 ASHG Annual Meeting

The role of cis- and trans-acting factors in aberrant *IKAROS* splicing in childhood leukemia. *P.A. Goodman, C.M. Wood, B.D. Juran, L. Sun, M.L. Crotty, F.M. Uckun.* Molecular Genetics, Parker Hughes Cancer Ctr/HI, St Paul, MN.

Ikaros (IK) is a zinc finger-containing transcription factor required for normal lymphocyte development, and germline mutant mice expressing only non-DNA binding dominant-negative isoforms of Ikaros develop an aggressive form of lymphoblastic leukemia between three and six months of age. Elevated expression of alternatively spliced dominant-negative Ikaros isoforms, lacking the DNA domain, as well as Ikaros isoforms with an in-frame deletion of 10 amino acids (D KSSMPQKFLG) have been observed in primary leukemic cells from children with acute lymphoblastic leukemia (ALL) (Proc. Natl. Acad. Sci. USA 96:680-685, 1999). The 10 amino acids involved in this deletion are encoded by the 3' end of exon 6, upstream of the transcription activation domain. Subsequent amplification and genomic sequence analysis of the corresponding exon 6-exon 7 splice junction regions from leukemic cells expressing the deletion variant demonstrated no mutation in the region spanning the cryptic splice site, or the predominant 5' (donor) or 3' (acceptor) splice sites. We then determined whether the deletion was mono- or biallelically expressed. A single nucleotide polymorphism (SNP) was identified affecting the third base of the triplet codon for a proline (CCC or CCA) in the highly conserved bipartite activation region (i.e., A or C at pos. 1002, from the translation start site of IK1). Biallelic expression of deletion and dominant-negative isoforms, along with wild-type isoforms, were observed in leukemic cells suggesting a trans effect on splice site selection. In compiling data from all leukemic clones sequenced (n=128), we observed an expression frequency of 77% 1002 ^{C} allele and 23% 1002 ^{A} allele. We also observed an excess of IK non-DNA binding isoforms (D KSSMPQKFLG) (91% C/9% A) on the C allele, as well as an excess of clones expressing the DNA binding isoforms (D KSSMPQKFLG) on the A allele (42% C/58% A). These results suggest a more subtle cis-acting influence on splice site recognition. Further detailed studies are required to determine the exact influence of both cis- and trans-acting elements on aberrant *IKAROS* expression in childhood ALL.

Program Nr: 684 from the 1999 ASHG Annual Meeting

MEN-1(Burin): Update on a unique phenotypic variant. *J.S. Green, C. Rigatto, P.S. Parfrey, S.M. Kaiser, A.B. Galway, C.J. Joyce, E. Ur.* Departments of Medical Genetics and Medicine, Memorial University, St. John's, NF, Canada.

BACKGROUND: We have previously described four families affected with MEN-1 originating in the Burin Peninsula of Newfoundland. Initial studies suggested a different tumor pattern than observed in other families. In 1997, the same mutation of the MEN-1 gene was identified in all four families. The present study integrates the results of symptomatic presentation, a decade of prospective clinical screening and genotyping in an updated description of this unique variant. METHODS: Prospective and retrospective outcomes assessment, genetic testing and prospective clinical screening (serum calcium, PTH, prolactin, gastrin, abdominal ultrasonography, chest x-ray) of those at 50% or greater risk of disease. Numbers in brackets represent 95% confidence intervals. RESULTS: Data was collected for 199 patients, of whom 165 had genetic testing. 95 of those tested were positive for the MEN-1 mutation, of whom 78 had clinical or biochemical evidence of disease and 17 did not. 70 patients tested negative for the gene. Overall, 94[89,99]%; of clinically affected patients developed hyperparathyroidism, 34[25,43]% developed pituitary tumors (32% PRL, 2% other), 10[4,16]% developed pancreatic tumors (4.3% gastrinoma, 3.3% glucagonoma, 2.3% other), 9[3,15]% developed carcinoid (5.3% lung, 3.3% thymus), and 3[0,6]% developed adrenal lesions (1% tumor, 2% multinodular disease). The initial feature of MEN-1 was hyperparathyroidism in 88[82,94]%, pituitary adenoma in 10[4,16]%, and carcinoid in 2[0,8]%. The penetrance of the disease was 50[39,61]% at age 34, 80[71,89]% at 45 years and 90[83,97]% at 51 years. Median age at diagnosis was 33[29,37] years for hyperparathyroidism, 37[27,45] for pituitary adenomas, 37[32,42] for pancreatic tumors and 42[38,48] for carcinoid. These differences were not statistically significant. CONCLUSIONS: MEN-1 (Burin) is characterized by a higher frequency of pituitary and carcinoid tumors, a lower frequency of pancreatic islet cell tumors, and a later onset than classical MEN-1. Since the same gene is involved as MEN-1 in other families, future studies need to focus on genetic and environmental variables that may influence phenotypic expression.

Program Nr: 685 from the 1999 ASHG Annual Meeting

Targeting of the p16/Rb pathway by methylation results in up-regulation of the p14ARF/p53 pathway in uveal melanoma. *N.A. Gruis[1], J.A.W. Metzelaar-Blok[2], P.A. van der Velden[1], H.M.H. Hurks[2], R.R. Frants[1], M.J. Jager[2]*. 1) Dept Human Genetics, Leiden Univ Medical Ctr, Leiden, Netherlands; 2) Dept of Ophthalmology, Leiden Univ Medical Ctr, Leiden, Netherlands.

The p16 (CDKN2A) gene on chromosome 9p21 encodes for the recognized tumor suppressor protein p16 and for a second protein product p14ARF, which originates from an unrelated exon of p16 (exon 1b) spliced onto exon 2 in an alternative reading frame. By means of a competitive RT-PCR, p16 and p14ARF mRNA expression was quantitatively determined in 9 uveal melanoma cell lines. Five out of nine cell lines lacked p16 expression and showed elevated p14ARF mRNA expression. The absence of p16 mRNA expression turned out to be due to hypermethylation of the p16 promoter. Treatment of the hypermethylated cell lines with the agent 5'-aza-2'-deoxycytidine led to re-expression of p16 and reduction of p14ARF mRNA expression. The inverse correlation between p16 and p14ARF mRNA expression might be explained by the interaction between cell cycle control pathways in which p16 and p14ARF play their roles. P16 induces a G1 cell cycle arrest by inhibiting the phosphorylation of retinoblastoma protein (Rb) by the cyclin-dependent kinases, CDK4 and CDK6. If p16 is repressed, phosphorylation of Rb results in the release of E2F transcription factor that stimulates transcription of p14ARF and the related p53-MDM2 pathway. The role of p16 hypermethylation in uveal melanoma was substantiated in primary tumors of which 27% showed p16 methylation, confirming that p16 promoter hypermethylation is a preferential mechanism of p16 inactivation.

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Program Nr: 686 from the 1999 ASHG Annual Meeting

The Association of Chromosome 8p Deletion and Tumor Metastasis in Human Hepatocellular Carcinoma. *X.-Y. Guan[1], L.-X. Qin[2], Z.-Y. Tang[2], J.S.T. Sham[1], Z.-C. Ma[2], S.-L. Ye[2], X.-D. Zhou[2], Z.-Q. Wu[2], J.M. Trent[3]*. 1) Clinical Nocology, University of Hong Kong, Hong Kong, P.R.China; 2) Liver Cancer Institute, Shanghai Medical University, Shanghai, P.R. China; 3) Cancer Genetics Branch, NHGRI, NIH, Bethesda, MD.

To understand the genetic mechanisms underlying the progression of hepatocellular carcinoma (HCC) metastasis, differences of genomic alterations between 10 pairs of primary HCC tumors and their matched metastatic lesions were analyzed by comparative genomic hybridization (CGH). Several genomic alterations including loss of 8p, 4q, 17p, and 19p, gain of 5p and high-level amplification of 1q12-q22 were detected in two or more cases. The most significant finding is the loss of 8p which was detected in 8 metastatic tumors but only in 3 corresponding primary tumors (p=0.03). This result suggests that the deletion of chromosome 8p might contribute to the development of HCC metastasis. Another interesting finding is the detection of a minimum amplification region at 1q12-q22 in HCC. This result provides a candidate amplification region in HCC for further study to identify amplified oncogenes related to the development or progression of HCC. Finally, this study provides a practicable model to detect specific genetic alterations related to the tumor metastasis through comparing the primary tumor and its corresponding metastatic lesion using CGH technique. Finally, this study provides a practicable model to detect specific genetic alterations related to the tumor metastasis through comparing the primary tumor and its corresponding metastatic lesion using CGH technique.

Program Nr: 687 from the 1999 ASHG Annual Meeting

Different rates of chromosomal changes as measured by comparative genomic hybridization in colon tumors with or without the CpG island methylator phenotype. *B.R. Haddad[1], J. Rone[1], M. Toyota[2], JPJ. Issa[2]*. 1) Institute for Molecular and Human Genetics, Georgetown Univ Medical Ctr, Washington, DC; 2) The Johns Hopkins Oncology Center, Baltimore, MD.

Tumors of the colon arise through a combination of genetic changes (mutations in APC/beta Catenin, K-RAS, p53, TGFbetaRII etc.), genetic instability (microsatellite or chromosome) and epigenetic changes (silencing and methylation of genes such as p16, hMLH1, THBS1, TIMP3, MGMT). A hypermethylator phenotype has recently been described in colon tumors termed CpG Island Methylator Phenotype (CIMP) which results in the simultaneous methylation and silencing of many genes. CIMP affects about half of all cases and is associated with a high rate of K-RAS mutations and a low rate of p53 mutations. We have now studied whole chromosomal changes by Comparative Genomic Hybridization (CGH) in a panel of 24 tumors also typed for CIMP and mutations in K-RAS and p53. 12 tumors had at least one alteration by CGH (4/11 adenomas and 8/13 carcinomas), and 8 tumors had two alterations or more. The most common changes were +20 or +20q (9/24 cases) and -18 or -18q (5/24 cases). Chromosomal changes were more common in CIMP Negative cases: 8/12 CIMP- tumors vs. 4/12 CIMP+ tumors had at least one change. 2/12 CIMP+ tumors had more than one alteration vs. 6/12 CIMP- cases. There were no correlations with K-RAS mutations or p16 methylation, but 7/8 tumors with p53 mutations had chromosomal changes vs. 5/16 tumors without such mutations. Thus, CIMP defines two groups of colon cancers with significantly different genetic changes as well as chromosomal changes as measured by CGH.

Program Nr: 688 from the 1999 ASHG Annual Meeting

The tumor antigene HuR binds specifically to one of five protein-binding segments in the 3'-untranslated region of the neurofibromin messenger RNA. *J. Haeussler[1], J. Haeusler[2], A. Striebel[2], G. Assum[2], W. Vogel[1], W. Krone[2]*. 1) Department of medical genetics, University of Ulm, Ulm, Germany; 2) Department of human genetics, University of Ulm, Ulm, Germany.

Neurofibromatosis type 1 (NF1) is one of the most common genetic disorders in humans, with a prevalence of about 1 in 4000 individuals. The NF1 gene spans about 335 kb genomic DNA and is organized in 60 exons that encode a transcript of 12.1 kb with a largest open reading frame of about 8600 bp. Neurofibromin, the gene product of the NF1 gene, belongs to the family of RAS GTPase activating proteins and has been shown to downregulate Ras activity. Interestingly, the large 3.5 kb 3'-untranslated region (UTR) of the NF1 mRNA shows significant sequence identity between species. 3'-UTRs have been found to affect the function of mRNAs in several ways, including intracellular localiziation of mRNAs, controlling mRNA stability and regulating translation efficiency. Because our knowlege about a specific function of the 3'-UTR of the NF1-mRNA is still in its infancy, we decided to search in this highly conserved structure for protein binding sites. We discovered five RNA fragments (NF1-PBR1-5, NF1-protein binding regions) that were able to undergo specific binding to proteins from cell lysates. In an initial effort to characterize the corresponding binding partners we identified the Elav-like protein HuR, an important component of the mRNA stabilsation machinery and a well known tumor antigene. The 32 kDA protein binds with high specificity to NF1-PBR1, a 38 nt fragment, located about 200 nucleotides downstream of the stop-codon. HuR has been found to interact with AU-rich elements (AREs) in the 3'UTR of many proto-oncogenes, cytokines and transcription factors, thereby regulating the expression of these messenger RNAs. Transient transfection assays with CAT reporter constructs containing or lacking NF1-PBR1 show that this sequence is able to inhibit expression of the reporter gene, indicating that HuR is involved in the fine tuning of the expression of the NF1 gene which codes for a protein functionally integrated into the complex network of Ras-mediated signal transduction.

Program Nr: 689 from the 1999 ASHG Annual Meeting

Structure and expression analysis of human and murine TCL loci. *C. Hallas, Y. Pekarsky, T. Itoyama, K. Huebner, C.M. Croce.* Kimmel Cancer Institute, Thomas Jefferson University, Philadelphia, PA.

The TCL1 and TCL1b oncogenes on human chromosome 14q32.1 are involved in the development of T cell leukemia in humans. Their expression in these leukemias is activated by chromosomal translocations and inversions involving 14q32 region. To investigate the possible involvement of other genes within this region in leukemia, we sequenced and characterized 110 kb human and 70 kb murine tcl loci. Analysis of ESTs, Northern and RT-PCR experiments revealed the presence of two novel human genes in the locus: TNG1 and TNG2 (for TCL neighboring genes 1 and 2). The protein sequences of these two genes do not show any homology in BLAST search. Expression profiles of all four human genes are very similar: all genes are expressed at very low levels in normal bone marrow, lymphocytes and B cell leukemia cell lines, but activated in T cell leukemias with rearrangements in 14q32 region. Surprisingly the 70 kb murine locus besides the tcl1 gene contains five other TCL1 related genes, called tcl1b1 through tcl1b5. The deducted amino acid sequences reveal very high similarity between these five genes suggesting that they are resulting from gene duplication. These genes show about 50 percent homology to the human TCL1b protein. All five genes are expressed in Knowles mouse two cell embryo cDNA library although at different levels and some of these genes are expressed at much lower levels in ES cells and some adult tissues. In contrast, the highest expression level of tcl1 was observed in ES cells and to a much lesser extent in two cell embryo cDNA library, suggesting differences in regulation of expression of murine tcl1 and tcl1b genes.

Program Nr: 690 from the 1999 ASHG Annual Meeting

Role of HMGI-C in familial lipomatosis. *R. Hamid, J.A. Phillips III, G.E. Tiller, J. Pfotenhauer.* Dept Pediatrics/Genetics, Vanderbilt Univ, Nashville, TN.

Lipomas (fat cell tumors) are one of the most common mesenchymal neoplasias in humans. Chromosomal translocations at 12q13-15 in sporadic lipomas have been described which disrupt the HMGI-C (high mobility group protein isoform 1-C) gene and fuse its DNA binding domains to novel transcriptional regulatory domains. This suggests that deranged HMGI-C expression may play a role in the pathogenesis of lipomas. To determine the genetic basis of familial lipomatosis and the role played by the HMGI-C gene, we have ascertained, examined and collected samples from members of a multiplex family with three affected generations with multiple lipomas. Aliquots of DNA from individual family members were used to genotype an intragenic dinucleotide repeat polymorphism within the HMGI-C gene. PCR analysis was done using fluorescently labeled primers and analyzed on an ABI 310 using Genotyper Software. We calculated a LOD score of -5.1 at theta = 0 from our data. These results show that the familial lipomatosis phenotype does not co-segregate with a particular allele of the HMGI-C gene or with sequences within 1 centi Morgan (cM) of the HMGI-C locus in this pedigree. There are additional positional candidate genes (CHOP, SAS, CDK4, MDM2) which localize to 12q but map to greater than 3 cM from the HMGI-C locus. In ongoing studies we are evaluating co-segregation of the above mentioned positional candidate genes to identify the familial lipomatosis locus in our kindred.

Program Nr: 691 from the 1999 ASHG Annual Meeting

Detecting leukemia specific translocations with a RT-PCR-ELISA method. *J. Han[1], S. He[1], D. Venable[1], P. Li[2].* 1) Genaco Biomedical Products, Inc., Huntsville, AL; 2) University of Alabama at Birmingham, Birmingham, AL.

Recurrent chromosome translocations in childhood leukemia are useful cytogenetic markers for monitoring diagnosis, prognosis and treatment. A traditional cytogenetic study, performed on patients' bone marrow cells, is a time consuming and labor intensive procedure that also requires extensive training of the cytogenetic technicians. We are developing a proprietary technology that will allow high throughput detection of multiple translocations at a time. The key feature of the technology is to perform a multiplex RT-PCR and hybridize the PCR products with capture probes covalently linked to ELISA plate. After post-hybridization washes, the captured PCR product is detected by a probe specific to either gene A or gene B that involved in the translocation. A translocation is detected if a microwell containing the capture oligo for gene A is detected by a probe specific for gene B. The method may represent a significant improvement for leukemia diagnosis, it will allow the screening of multiple translocations at a time, and the whole procedure can be completed within hours.

Program Nr: 692 from the 1999 ASHG Annual Meeting

Genomic sequence analysis and SNP identification in genomic regions surrounding genes involved in carcinogenesis. *S.L. Haydel, R.A. Gibbs, D.L. Nelson.* Dept Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Numerous genes have been identified that confer higher risk of development of cancer when mutated. The role of common variants of these genes in conferring elevated (or diminished) risk of cancer is unknown. This project seeks to identify functional variants of genes involved in replication, repair or cell cycle regulation and to develop tools for performing association studies in sporadic cancer. We are determining the genomic sequence of large regions containing candidate genes. Human genomic BAC and PAC libraries are screened using overgo probes designed from cDNAs. A small contig is constructed by STS and restriction fingerprinting analysis, and a single large clone (~150kb) carrying the complete gene is sequenced by the BCM Human Genome Sequencing Center. To date, ten genes are in the sequencing process. These are *MSH6*, *MSH2*, *MSH3*,*MLH1*, *PMS1*, *P53*, *KU80/6*, *HRAS*, *DNA PKCS*and *RECQL*. The *RECQL* sequence is complete. It reveals a gene spanning approximately 30 kb and made up of 15 exons. Within this same BAC clone derived from 12p12 are also genes for islet amyloid polypeptide and glycogen synthase, as well as unknown genes represented by numerous EST similarities. Sequence data are used to identify variable nucleotides by resequencing randomly selected regions of the gene in five unrelated samples. Our goal is to develop 8-12 SNPs per locus in order to provide a rich haplotype for each. Allele frequencies and haplotype structures are determined for each locus by typing 300 ethnically diverse samples and 10 3 generation CEPH families. At present, 8 variable alleles have been identified from 20 sequencing products in the *BRCA2* locus, and allele specific oligonucleotide assays are underway for haplotype studies.

Program Nr: 693 from the 1999 ASHG Annual Meeting

Acute Lymphoblastic Leukemia (ALL) in Children with Down Syndrome (DS): A Report from the Children's Cancer Group (CCG). *N.A. Heerema[1], H.N. Sather[2], M.G. Sensel[2], M.K. Lee[2], B.J. Lange[2], B. Bostrom[2], J.B. Nachman[2], P.G. Steinherz[2], R. Hutchinson[2], P.S. Gaynon[2], F.M. Uckun[1].* 1) Hughes Inst., St. Paul, MN; 2) Children's Cancer Group, Arcadia, CA.

We identified 34 children with DS among 1880 children with ALL treated on risk-adjusted CCG protocols. Fifteen patients (44%) had no cytogenetic abnormalities other than a constitutional (c) +21; ten patients (32%) had 1 or 2 additional abnormalities; five patients (24%) had complex karyotypes with more than two additional abnormalities but with a modal chromosome number (MN) <51; four patients had high hyperdiploidy (MN >51). Known recurring additional cytogenetic abnormalities included an abnormal 9p (3 patients); an abnormal 12p (5 patients); a 14q32 breakpoint (2 patients); an 11q23 breakpoint (1 patient); a +X with modal number <51 (2 patients); and an i(17)(q10) in 2 patients. No DS patients had a t(4;11), a t(1;19), or a t(9;22). DS patients were less likely than non-DS patients to have high white blood cell (WBC; p = 0.02), high platelet counts (p = 0.04), or significantly enlarged lymph nodes. DS patients were more likely to be classified as standard risk (age 1-9 years with WBC <50,000/mL; p = 0.01), but were somewhat less likely to have a rapid response to initial therapy (<25% blasts on day 7; p = 0.10). The majority of patients with or without DS had B-lineage ALL. Event-free survival (EFS) was similar for DS and non-DS patients, with 6-year estimates of 76% (SD=11%) and 75% (SD=2%), respectively (p=0.93). DS patients experienced events within two years of study entry, whereas non-DS patients had fewer early events and more later events. There was a trend for decreased overall survival for DS patients: 6-year estimates were 76% (SD=11%) and 83% (SD=1%), respectively (p=0.17; relative risk=1.64). Four DS patients' first events were toxic deaths; 3 of the 4 patients with other first events also subsequently died of toxicities. These data suggest that with current therapy, children with DS who develop ALL have outcome similar to that of non-DS patients, although the temporal sequence and types of events differ from the those of the non-DS ALL population.

Program Nr: 694 from the 1999 ASHG Annual Meeting

Expression of $p16^{INK4a}$ and $p19^{ARF}$ in squamous cell carcinoma cell lines. *T.N. Heffernan[1], M.F. Shaheen[2], D.T. Cody[1]*. 1) Surgery, SIU School of Medicine, Springfield, IL; 2) Internal Medicine, SIU School of Medicine, Springfield, IL.

$p16^{INK4a}$ and $p19^{ARF}$ ($p14^{ARF}$, p16b) are located on the short arm of chromosome 9 and share the same locus at 9p21. However, the two transcripts have different first exons and the shared second and third exons have alternate reading frames so that the two products lack amino acid identity. Both $p16^{INK4a}$ and $p19^{ARF}$ encode proteins that function in cell cycle regulation. $p16^{INK4a}$ is a cyclin-dependent kinase (CDK) inhibitor that regulates the Rb pathway. It acts by inhibiting CDK4 and CDK6 from binding to cyclin D, thereby causing Rb to be hypophosphorylated so that the cell cycle ceases or is controlled at the G_1/S phase. On the other hand, $p19^{ARF}$ regulates the p53 pathway. It acts by binding to MDM2, thereby inhibiting MDM2 from targeting p53 for ubiquination and degradation so that p53 levels are stabilized and the cell cycle is arrested or controlled at both the G_1/S and the G_2/M phase. Since the short arm of chromosome 9, where $p16^{INK4a}$ and $p19^{ARF}$ are located, is frequently mutated in human cancers, and $p16^{INK4a}$ and $p19^{ARF}$ regulate the cell cycle, expression levels of these genes are ideal targets for study in various cancer cell lines.

We used Western blot analysis to determine expression levels of these genes in the squamous cell carcinoma cell line, SCC-9 (ATCC). The Saos-2 (ATCC) cell line was used as a positive control and normal adult human epithelial keratinocytes (HEKa, Cascade Biologics) were used to determine baseline expression levels. The SCC-25 (ATCC) cell line was used as a negative control.

We were able to demonstrate that the Saos-2 cell line and the HEKa cells both expressed $p16^{INK4a}$ and $p19^{ARF}$; whereas, the SCC-25 cell line, as expected, showed no detectable expression. Using this assay, the SCC-9 cell line did not show expression of either $p16^{INK4a}$ or $p19^{ARF}$. Lack of detectable expression of $p16^{INK4a}$ and $p19^{ARF}$ in the SCC-9 and SCC-25 cell lines may contribute to the uncontrolled cell cycling that leads to squamous cell carcinoma development and supports the idea that inactivation of these genes contributes to carcinogenesis.

Program Nr: 695 from the 1999 ASHG Annual Meeting

Molecular cytogenetic characterization of a highly aberrant plasmocytoma case with 24-color FISH, CGH, multicolor banding (MCB) and region specific FISH probes. *A. Heller[1], H. Starke[1], I.F. Loncarevic[1], G. Senger[2], I. Chudoba[3], U. Claussen[1], T. Liehr[1]*. 1) Inst. Human Genetics and Anthropology, Jena, Germany; 2) Practice of Medical Genetics and Gynaecology, Regensburg, Germany; 3) MetaSystems Inc., Altlussheim, Germany.

A case of a 72 year old female patient suffering from plasmocytoma is reported. In conventional cytogenetic analysis (GTG-banding) a highly complex karyotype with 51 to 53 chromosomes and at least 2 different subclones could be identified. Trisomy 3, 5, 7, 14, 15 and 19 could be observed by GTG-banding and confirmed by 24-color FISH. CGH revealed the following changes: rev ish enh(3,5p,7,8,9,15,19p),dim(1p31p11,13,14q21q31), amp(1q12-q44,5p15.3q15,Xq21q28). Moreover, the involvement of chromosome 1 in complex rearrangements was studied more intensely using the DNA-based high resolution multicolor-banding technique (MCB), based on changing fluorescence intensity ratios along the chromosomes (Chudoba et al., Cytogenet. Cell Genet., in press). Using an MCB probe set for chromosome 1, a partial trisomy 1p11-qter, a partial monosomy 1p11-p31.1, a translocation t(1;7)(p13.3;?) and an inversion p13.3-q21.1 could be identified. An MCB probe set for chromosome 5 and 13 has also been used and revealed complex rearrangements in this case. FISH using a p53 specific probe revealed a heterozygous deletion in 38% of the evaluated nuclei, even though - according to GTG banding - both chromosomes 17 appeared to be normal. Complementary studies using region specific cosmid probes (RB1 on 13q14, AML1 on 21q22, Int2 on 11q13 and MLL on 11q23) are in progress to characterize the highly complex changes of this case in more detail. Acknowledgments: This work was supported by the Madeleine Buehler-Kinderkrebs-Stiftung and a fellowship to A. Heller and H. Starke by the Herbert Quandt Stiftung der VARTA AG.

Program Nr: 696 from the 1999 ASHG Annual Meeting

Reduced expression of dystroglycan in prostate and breast cancer. *M.D. Henry[1], M.B. Cohen[2], M. Durbeej[1], K.P. Campbell[1]*. 1) HHMI, Department of Physiology and Biophysics, Neurology, University of Iowa, Iowa City, IA; 2) Department of Pathology, University of Iowa, Iowa City, IA.

Cellular interactions with the extracellular matrix are an important aspect in the development and progression of many types of cancer. We have elucidated a central role for dystroglycan, a cell surface receptor for several extracellular matrix proteins, in the organization of the laminin-based extracellular matrix during development. Since abnormalities of the laminin-based matrix are hallmarks of metastatic disease, we examined the status of dystroglycan expression in prostate tumors. We examined 15 cases of surgically resected prostate tumors by immunofluorescence analysis to detect the dystroglycan protein. We find a marked reduction of dystroglycan expression in malignant tissues compared to normal prostatic epithelia. This difference was most pronounced in high grade disease. Similar findings were made for mammary ductal adenocarcinomas suggesting that loss or reduction of dystroglycan expression may be a conserved feature of epithelial neoplasia. In conclusion, these data suggest that reduced expression of dystroglycan in prostate and breast may contribute to the abnormalities in the laminin-based extracellular matrix observed in neoplastic diseases, and may therefore contribute significantly to cancer cell invasion and metastasis.

Program Nr: 697 from the 1999 ASHG Annual Meeting

hMLH1 and hMSH2 mutation analysis in 112 suspected HNPCC families. *E. Holinski-Feder[1], Y. Mller-Koch[1], B. Neitzel[1], R. Kopp[3], P. Lohse[2], M. Gross[5], G. Baretton[4], B. Kerker[1], Ch. Neuschfer[1], A. Wirtz[1], J. Murken[1]*. 1) Dep. Medical Genetics; 2) Inst. of Clinical Chemistry; 3) Dep. of Surgery; 4) Dep. of Pathology; 5) Dep. of Gastroenterology, University of Munich, Germany.

To determine the pattern and incidence of MSI status and mutations in the HNPCC genes MLH1 and MSH2 in the german population, 107 families were divided in risk groups according to Amsterdam I/II- (19 families), Bethesda-criteria (36 families) and 51 families with suspected hereditary colorectal cancer, missing the criteria above. Mutation analysis was speeded up by implementing an innovative and cost efficient method called denaturing high-performance liquid chromatgraphy (DHPLC) on the equipement supplied by TRANSGENOMICS. The power of the method resides in its sensitivity and ability to rapidly and reproducably compare amplified sequnces in an automated manner.The analysis conditions for each exon were determined on the basis of the exon specific melting profiles and the software programme WAVEMAKER. Fullfillment of the Amsterdam I criteria is associated with an increased number of MSI-H tumors (13/17) and a increased mutation detection rate (10/24). 36 families fulfilling the Bethesda criteria showed 6 MSI-H tumors and 3 mutations. 51 families not matching the criteria above showed 4 MSI-H tumors and 1 mutation. Two mutations, one published and one unpublished, were found twice in unrelated families pointing towards founder mutations in the german population. To exclude attenuated FAP in these and the other families, mutation analysis of the exons 1-4, 9 of the APC-gene was performed for 75 patients. We found one mutation in exon 4 in one patient out of the Amsterdam I cohort. For the mutation negative Amsterdam I families the hMSH6 gene is currently screened. These results demonstrate the highest incidence of mutations in Amsterdam I families, they underscore the importance of MSI analysis prior to mutation analysis in high risk, but also in low risk families. One third of the Amsterdam I families show MSI-S tumors and are not caused by mutations in hMLH1 or hMSH2 and one familiy would have been missed even by the loose Bethesda criteria.

Program Nr: 698 from the 1999 ASHG Annual Meeting

Microsatellite instability and MLH1 hypermethylation in endometrial carcinomas of the endometrioid subtype.

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Microsatellite instability (MSI) is a common phenotype of many sporadic tumours and tumours associated with hereditary non-polyposis colorectal cancer (HNPCC). MSI indicates a mutator phenotype due to mutation in genes such as the DNA mismatch repair genes which result in genomic instability. This is hypothesized to accelerate the accumulation of genetic alterations that occur in the development of neoplasia. MSI is found in 17-32% of all sporadic endometrial carcinomas, depending on the samples tested and the assay used. Very few studies have tried to correlate MSI status with clinicopathologic features and findings are contradictory due to the small numbers of studies and the lack of uniformity in MSI detection methods and in case selection. We have undertaken a study of 100 endometrial carcinomas of the endometrioid subtype (UECs) which account for 90% of all endometrial carcinomas. These have been reviewed and selected on the basis of varying grade of tumour and degree of invasion of the myometrium and cervix by a single gynecological pathologist. We used the BAT-26 marker, a highly efficient (99%) marker of MSI to accurately determine the proportion of UECs that demonstrate MSI. Genetic findings are correlated with pathologic findings and the final interpretation includes MSI positivity or negativity, associated genetic changes in the MSI+ cases and the correlation of MSI status with pathologic features of the carcinoma. Recently, it has been shown that up to 90% of MSI+ endometrial tumours demonstrate inactivation of the mismatch repair gene MLHI by epigenetic hypermethylation. The MSI+ tumours in this study will be used to determine the role of MLH1 hypermethylation in this cohort, and if there is any correlation with tumour grade or invasiveness. This will help determine how important epigenetic methylation is in the development of endometrial carcinoma. This sets the stage for studies with additional molecular markers specific to UECs lacking DNA mismatch repair that may prove useful for prognosis and gene therapy.

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Program Nr: 699 from the 1999 ASHG Annual Meeting

Population-based estimate of the average age-specific cumulative risk of breast cancer for a defined set of protein-truncating mutations in BRCA1 and BRCA2. *J.L. Hopper[1], M.C. Southey[2], G.S. Dite[1], D.J. Jolley[1], G.G. Giles[3], M.R.E. McCredie[4], D.F. Easton[5], D.J. Venter[2]*. 1) Centre for Genetic Epidemiology, The University of Melbourne, Carlton, Victoria, Australia; 2) Peter MacCallum Cancer Institute, Melbourne, Victoria, Australia; 3) Anti-Cancer Council of Victoria, Carlton, Victoria, Australia; 4) New South Wales Cancer Council, Woolloomooloo, New South Wales, Australia; 5) CRC Epidemiology Unit, University of Cambridge, Cambridge, United Kingdom.

The average breast cancer risk for carriers of a germline mutation in BRCA1 or BRCA2 (penetrance) has been estimated - from the multiple-case families collected by the Breast Cancer Linkage Consortium (BCLC) - to be about 80% to age 70. However, women now being tested for these mutations do not necessarily have the intense family history of the BCLC families. Testing for protein-truncating mutations in exons 2, 11 and 20 of BRCA1, and exons 10 and 11 of BRCA2, was conducted in a population-based sample of 388 Australian women with breast cancer diagnosed before age 40. Onset of breast cancer was analysed in the known, and potential, mutation-carrying first and second degree female relatives of cases found to carry a mutation. Of the 18 mutation-carrying cases (9 BRCA1, 9 BRCA2), only five (1 BRCA1, 4 BRCA2) had at least one affected relative, so family history of breast cancer was not a strong predictor of mutation status in this setting. The risk in mutation-carriers was on average 9 (C.I. 4-23) times the population risk ($p < 0.001$). Penetrance to age 70 was 40% (C.I. 15%-65%), about half that estimated from BCLC families ($p < 0.05$). Extrapolating, about 6% (C.I. 2%-20%) of breast cancer before age 40 may be caused by protein-truncating mutations in BRCA1 or BRCA2. Breast cancer risk in BRCA1 or BRCA2 mutation carriers may be modified by other genetic, or environmental, factors. Genetic counselling may need to take into account the family history of the consultand.

Program Nr: 700 from the 1999 ASHG Annual Meeting

Unusual presentation of desmoid tumors in a family with Familial Adenomatous Polyposis. *C.L. Hunter, G.H. Vance.* Dept Medical & Molecular Gen, Indiana Univ, Indianapolis, IN.

Desmoid tumors are rare fibrous tumors occurring at increased frequency in Familial Adenomatous Polyposis (FAP). FAP is an autosomal dominantly inherited condition resulting from genetic mutations in the Adenomatous Polyposis Coli (APC) gene and predisposing to hundreds of adenomatous colonic polyps. A subset of families with FAP develop extra-colonic features such as desmoid tumors, osteomas, congenital hypertrophy of the retinal pigment epithelium, and supernumerary teeth. It is estimated that individuals with FAP have an 852-fold increased risk for developing a desmoid tumor (Gurbutz, et. al., 1994). Typically, FAP-associated desmoid tumors develop abdominally and either at the time of or following a diagnosis of FAP, i. e.: after polyposis or multiple adenomatous colonic polyps have developed. The average age of FAP-associated desmoid tumor development reported in various FAP registries ranges from the late twenties to early thirties.

We report on a family with a diagnosis of FAP and an unusual presentation of desmoid tumors. The proband presented at age 25 years with a desmoid tumor of the breast and no family history of colon cancer, FAP or of desmoid tumors. During the following 7 years, multiple abdominal and retroperitoneal desmoid tumors as well as multiple adenomatous polyps developed. The proband's daughter presented to her pediatrician at 10 days of age with an infantile (desmoid-like) fibromatosis tumor on her left lower leg. At age 10 years she underwent her first colonoscopy which identified several adenomatous polyps. The third affected family member is the proband's son who underwent his first colonoscopy at age 12 years during which several adenomatous polyps were identified. He has not developed a desmoid tumor. Commercial genetic testing has identified a truncated APC protein in all three individuals. The atypical features seen in this family include 1) two of the three affected family members developing a desmoid tumor as the initial manifestation of their disease, 2) the initial desmoid tumors developing extra-abdominally, and 3) the unusually young age of desmoid tumor development seen in one individual.

Program Nr: 701 from the 1999 ASHG Annual Meeting

Impact of smoking habit and germline p53 mutation on cancer risk. *S. Hwang[1], B.M. Mims[2], G. Lozano[2], L.C. Strong[1]*. 1) Dept Experimental Pediatrics, Univ TX MD Anderson Cancer Ctr, Houston, TX; 2) Department of Molecular Genetics, University of Texas M. D. Anderson Cancer Center, Houston Texas.

The accumulated studies have clearly indicated cigarette smoking as a major risk factor for various cancers in the general population. The unique mutation spectrum of the tumor suppressor gene p53 in smoking-related tumors further strengthened the relationship. It is unknown, however, how cigarette smoking affects the cancer risk in individuals with an inherited p53 mutation. In a familial cohort systematically ascertained through childhood sarcoma patients largely characterized by p53 mutation status, we evaluated the impact of cigarette smoking in smoking-associated-cancer (SAC, including cancers in lung, larynx, oral cavity, and esophagus) risk. The study population included relatives of 540 childhood sarcoma patients, including 44 germline p53 mutation carriers in 10 kindreds, and 4983 relatives in 530 kindreds without evidence of a germline p53 mutation. Association between cigarette smoking and cancer was evaluated using the Cox's proportional hazard models. Smoking was not associated all types of cancer risk in the carriers but was significantly associated with cancer risk in the non-carriers. After adjusted the effects of birth year, sex, and race, we observed statistically significant likelihood change for SAC after adding the effect of cigarette smoking in both models for carriers and non-carriers. Our results revealed a hazard ratio of 4.74 in the non-carriers and a hazard ratio of 10.76 in the carriers for cigarette smoking and SAC risk. Interaction between smoking and carrying germline p53 mutation in cancer risk was not statistically significant. The findings suggest that inheritance of a p53 mutation dramatically increases the risk of SAC, and that the risk is further increased by cigarette smoking.

Program Nr: 702 from the 1999 ASHG Annual Meeting

Molecular analysis of chromosome 6p rearrangement in Retinoblastoma. *I.M. IMBERT[1,2], O. JAFER[2], L.J. COIGNET[2], F. PELLESTOR[1]*. 1) Institute of Human Genetics, CNRS UPR 1142, Montpellier, France; 2) Institute of Cancer Research,Royal Marsden Hospital, Sutton, UK.

Recurrent cytogenetic abnormalities are the hallmark of all malignant tumors. Classification of malignancies depends on the location of the chromosomic disruption/deletion, which relates to prognosis of the disease and therapeutic choices. Molecular genetics and cytogenetics techniques are used to identify oncogenes localized near the chromosomic breakpoints, and oncogenes activation seem to be dependent on translocation mechanism. Recurrent atypical cytogenetic abnormalities of chromosome 6p have been reported in a few cases of translocations implicated in Retinoblastoma. The translocation t(4;6)(p15;p21.2) was studied in the Y79 cell line established from the primary tumor (right eye) of a 2-year-old caucasian girl in 1971. To determine the site of the breakpoint on 6p, yeast artificial chromosome (YAC) clones from p21 to p22 bands were used. The breakpoint has been localised by FISH on 6p21.3 and the TNF-alpha gene has been shown to be involved in this rearrangement. TNF-alpha is a cytokine, a specialized hormone-like protein that can influence cellular development and function. To measure the expression of TNF-alpha gene in retinoblastoma cells, flow cytometric analysis and cytospins were performed using an anti-TNF-alpha antibody. This study has shown an enhancement of the expression of TNF-alpha gene in Y79 cells. Further cloning, sequencing and characterization of the gene(s) involved in this rearrangement from patient samples with this recurrent breakpoint will allow us to study the possible consequences of disruption/deletion of this gene in the development or progression of the disease.

Program Nr: 703 from the 1999 ASHG Annual Meeting

HER2/neu and cMYC alterations in breast lesions from women with and without a family history of breast cancer. *R.B. Jenkins, C.A. Reynolds, C.A. Soderberg, D.C. Melder, F.J. Couch, L.C. Hartmann.* Mayo Clinic, Rochester, MN.

The biology of breast cancer initiation and progression is poorly understood. Germline mutations in BRCA1, BRCA2, and other susceptibility loci are involved in the initiation of hereditary breast cancer. However, the somatic events that lead to progression of hereditary cancers are not known. In addition, it is not known whether somatic progression pathways differ in hereditary versus sporadic disease. In order to evaluate these questions, we have developed a registry of >500 women who underwent both therapeutic mastectomy to remove a cancer as well as a contralateral prophylactic mastectomy. At present, we have studied 22 hereditary cases and 29 matched sporadic cases from this cohort, with and without a strong family history of breast and/or ovarian cancer, respectively. For each case, paraffin blocks were available for both mastectomy specimens. Among these blocks, 46 in situ carcinomas and 51 invasive carcinomas were identified. These lesions as well as 32 regions of apparently benign epithelium (for control purposes) were subjected to fluorescent in situ hybridization (FISH) analysis using the HER2/neu/CEN17 and cMYC/CEN8 dual-probe mixtures. The incidences of oncogene amplification in hereditary and sporadic cases, respectively, were 28% (6 of 21) and 4% (1 of 27) for HER2/neu (p=0.04) and 19% (4 of 21) and 4% (1 of 27) for cMYC (p=0.09). The incidences of chromosomal gain in hereditary and sporadic cases, respectively, were 48% (10 of 21) and 11% (3 of 27) for +CEN17 (p=0.009) and 52% (11 of 21) and 26% (7 of 27) for +CEN8 (p=0.06). The incidences of any anomaly in hereditary and sporadic cases, respectively, were 67% (14 of 21) and 27% (7 of 26) (p=0.008). Our data show 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 separate pathways of carcinogenesis in hereditary versus sporadic disease.

Program Nr: 704 from the 1999 ASHG Annual Meeting

Genomic alterations in six bladder cancer cell lines studied by DNA arrays, comparative genomic hybridization, and flourescence in situ hybridization. *J.P. Johnson, H. Maruyama, S. DeVries, E. Gum, K. Golden, F.M. Waldman.*
Cancer Center, UC San Francisco, San Francisco, CA.

Comparative genomic hybridization has been able to identify many common sites of altered copy number in human cancers. In this study we used chromosome CGH to define chromosomal gains and losses in six commonly studied bladder cancer cell lines (J82, TCCSUP, HT1197, HT1376, 5637, and T24). In addition, FISH and array-based CGH were compared with chromosome-based CGH for utility in characterizing chromosome 20 gains at high resolution. The mean number of abnormalities seen by chromosome based CGH was 13.5 (range 4-25). All cell lines showed a gain involving chromosome twenty. Since gains in chromosome twenty have been associated with immortalization of urothelial cell lines, and with progression of primary tumors, the substructure of the chromosome 20 gain was further studied by fluorescence in situ hybridization (FISH) with a set of chromosome 20 probes, and by array-based CGH. These approaches yielded higher resolution than chromosomal CGH, showing the substructure of the 20q amplicon. Array CGH showed that 20q11 and 20q13.2 were consistently gained in all 20q amplified cell lines. Also, combining FISH and CGH approaches allowed for determination of absolute copy number by scaling of array-based ratios. Analysis of a series of oncogenes and tumor suppressor genes in these cell lines showed that array-based CGH was consistently able to detect deletions as well as DNA copy number gains. 20q gains were detected by chromosomal CGH in five of the six cell lines tested, while high level amplification of 20p was seen in the sixth, HT1197. Regions of chromosomes 6 and 11 were gained in five of the cell lines. 11p was consistently gained in all five, and amplification involving 6p22 was observed in four of the cell lines. Also, regions of chromosomes 4 and 8p were lost in five cell lines. Region 8p21-pter was the smallest common region of 8p loss, present alone in two of the cell lines. These data show that array based CGH analysis of chromosomal gains and losses are validated by alternative approaches, and are useful in defining alterations at high resolution.

Program Nr: 705 from the 1999 ASHG Annual Meeting

Characterization of a novel 17q25 GTPase gene suggests evidence for its role as a breast tumor suppressor gene.
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We have previously defined a 300 kb minimal region of allelic imbalance on 17q25 by loss of heterozygosity studies in breast and ovarian tumors (Kalikin, LM et al., 1996, 1997). We hypothesize that this interval harbors a novel tumor suppressor gene. Toward identification of this gene, transcripts were isolate by solution hybrid capture from BACs and P1s spanning the candidate region. A novel EST with strong homology to a GTPase family of genes involved in cytokinesis was isolated. This gene showed variable expression of 3.5 and 4.0 kb transcripts in most tissues tested. Preliminary results from breast tumor cell line Northern and Southern blots revealed potentially altered bands in some samples. Recently, this same gene was reported as part of a fusion protein in an acute myeloid leukemia patient with a t(11;17)(q23;q25) rearrangement (Osaka, M et al., 1999). As these observations suggest that the normal cellular role of this gene may be altered by both gain of function and loss of function mutations, further structural and expression characterization is important to understanding its potential role in tumorigenesis and leukemogenesis. Toward this end, we have more precisely defined the genomic structure of this gene including exon/intron boundaries and transcript variabilities at the 5' end. Mutational analysis is ongoing in breast tumor samples exhibiting distinct 17q25 minimal regions of allelic imbalance or altered bands on Northern or Southern blots.

Program Nr: 706 from the 1999 ASHG Annual Meeting

Familial medullary thyroid carcinoma and prominent corneal nerves associated with a codon 804 germline mutation of the *RET* gene. *L. Kasprzak[1], S. Nolet[2], L. Gaboury[2], W. Gregory[1], C. Pavía[4], C. Villabona[5], F. Rivera-Fillat[3], J. Oriola[3], W.D. Foulkes[1]*. 1) Department of Medicine, McGill University, Montreal, PQ, Canada; 2) Département de Pathologie, Laboratoire d'Oncologie et de Pathologie Moléculaire, Université de Montréal, PQ, Canada; 3) Servei d'Hormonologia, (IDIBAPS), Hospital Clínic, Barcelona, Spain; 4) Secció de Endocrinologia, Hospital Universitari de Sant Joan de Deu, Barcelona, Spain; 5) Servei d'Endocrinologia, CSU Bellvitge, Hospital Principes de España, Barcelona, Spain.

Germline mutations in the *RET* protooncogene are associated with Multiple Endocrine Neoplasia (MEN) type 2A, 2B and familial medullary thyroid carcinoma (FMTC). MEN2A is characterised primarily by medullary thyroid carcinoma (MTC) and pheochromocytoma. MEN2B has additional stigmata including a marfanoid habitus, mucosal neuromas and corneal nerve thickening. In FMTC, the only lesion present is MTC. Although characteristic of MEN2B, prominent corneal nerves have been noted in individuals with MEN2A.

We report a unique, four generation kindred in which three individuals had MTC. The proband and his mother had visible corneal nerves without other features of MEN2. The family was initially thought to represent a phenotype consistent with MEN2B. However, subsequent assessment at two centers (Canada and Spain) confirmed prominent corneal nerves in three family members and no other symptoms of MEN2B. Molecular genetic studies were performed on the two affected and six unaffected family members. Germline mutation in exon 14, codon 804 of the *RET* was identified in four individuals. Furthermore, all of the mutation carriers but none of the non-carriers were found to carry a previously unreported variant at codon 778 in exon 13.

The relatively rare mutation at codon 804 (exon 14) has previously only been seen in FMTC families. V778I variant cosegregates with the disease allele and perhaps could be contributing to the distinct phenotype present in this family.

Program Nr: 707 from the 1999 ASHG Annual Meeting

FISH studies of c-myc, cyclin-D1, p53 and chromosome 8, 11 and 17 centromeres in Barrett's, dysplasia and adenocarcinoma of the esophagus. *N. KATAOKA[1], S.R. Young[1], Z. Wang[1], J.W. Jakub[2]*. 1) 1Department of Obstetrics and Gynecology, University of South Carolina School of Medicine, Columbia, SC; 2) 2Department of Surgery, University of South Carolina School of Medicine, Columbia, SC.

Most esophageal adenocarcinomas are thought to arise from the metaplastic columnar epithelium (Barrett's esophagus) through dysplastic changes and a series of genetic alterations. It is perhaps generally accepted that early diagnosis of carcinoma is needed for optimal treatment and good prognosis. Recent studies have described tp53 overexpression and Her2/neu amplification as potential markers of esophageal carcinoma and its precursor. The oncogene c-myc is known to have an important role in early embryogenesis, cell-growth control, cell differentiation and the tissue-repair process. Cyclin-D1 is known to have a very important role in regulating the cell cycle. One of the most common tumor suppressor genes in human cancer is p53. The purpose of this study was to evaluate possible molecular cytogenetic changes in c-myc, cyclin D1 and p53 in esophageal disease. Eleven specimens of paraffin-embedded, formalin-fixed esophageal disease (three Barrett's esophagus, five mild dysplasia, one severe dysplasia, two adenocarcinoma) were obtained and studied for quantification of the oncogene c-myc relative to the centromere of chromosome 8, cyclin D1 relative to the centromere of chromosome 11 and p53 relative to the centromere of chromosome 17 using a combination of dual color FISH. All eleven samples showed normal findings of c-myc/#8 and p53/#17. However, one of the two adenocarcinoma specimens exhibited amplification of cyclin D1. In this limited study, it appears that p53 and c-myc are not associated with esophageal carcinoma progression, but cyclin D1 amplification may be involved in this progression.

Program Nr: 708 from the 1999 ASHG Annual Meeting

The inheritance of missense mutations in Ataxia-Telangiectasia (A-T) families. *N. Khanlou, R.A. Gatti.* Department of Pathology, UCLA School of Medicine, Los Angeles, CA.

Increased cancer susceptibility is one of the major characteristics of A-T, an autosomal recessive disorder. A-T heterozygotes are estimated to constitute 5% of all cancer patients before the age of 46 in the USA. Several studies have shown somatic loss of heterozygosity in the ATM region and there is conflicting evidence as to whether ATM can function as a tumor suppressor. Recent studies suggest a role for ATM missense mutations in cancer susceptibility, especially with regard to breast cancer. In one study, the standard incidence ratio of breast cancer in relatives of Scandinavian A-T patients was estimated at 7.7 for mothers, 1.6 for grandmothers and 1.7 for great-grandmothers. We were impressed by the unexpected high ratio in great-grandmothers and, considering the major role of ATM in meiosis and gametogenesis, we hypothesized that perhaps ATM missense mutations might be more frequently inherited from one parent or the other. The segregation of eight missense mutations was investigated by single strand conformation polymorphism. None of these mutations involved a splice site. None of the families had a history of breast cancer. Five mutations were inherited from the father; three were inherited from the mother. These results suggest that ATM missense mutations are randomly inherited from father or mother, with no sex bias.

Program Nr: 709 from the 1999 ASHG Annual Meeting

The expression of the PAX2 in human prostate cancer. *B. Khoubehi[1,2], C.W. Ogden[2], J.M. Adshead[1,2], A.M. Kessling[1]*. 1) Medical and Community genetics, Imperial College, London, Harrow, England; 2) Department of Urology, Northwick Park Hospital, Watford Road, London, HA1 3UJ.

Prostate cancer is the most common solid tumour in American men and is the second most common cause of cancer death. Despite high incidence and mortality rate, the molecular mechanisms underlying the oncogenesis and progression of the prostate cancer are still unclear.

PAX genes encode nine nuclear transcription factors, which are essential for embryogenesis and are proto-oncogenes in mice. The class III PAX genes (PAX2,5&8) are the most likely candidates for a role in oncogenesis due to the salient feature of their expression in highly mitotic, undifferentiated cells during development and their inappropriate expression in a number of human tumours. PAX2 is expressed at high levels in the developing undifferentiated cells of the urogenital system and is downregulated upon terminal differentiation with no expression in normal adult cells. We studied PAX2 expression in prostate cancer using reverse transcriptase PCR and found no expression in histopathologically confirmed benign prostatic hypertrophy specimens (0/10), whereas PAX2 was expressed in 3/3 established cancer cell lines and 10/27 (37%) primary prostate cancers. Expression of PAX2 in the cell lines was confirmed using immunofluorescence.

The Fishers exact test for comparison of PAX2 expression in prostatic specimens gave p= 0.024. These data suggest PAX2 expression in prostate cancer cells may contribute to pathogenesis by supporting cellular proliferation in the de-differentiated state.

Program Nr: 710 from the 1999 ASHG Annual Meeting

Gene Expression Profiles of Esophageal Cancer Patients Using cDNA Microarray Technology. *C. Kihara[1,2], T. Tanaka[1], Y. Furukawa[1], K. Ono[1], O. Kitahara[1], N. Shiraishi[1], R. Yanagawa[1], H. Ogasawara[1], T. Seki[1], H. Yamana[3], T. Tsunoda[4], K. Hirata[2], T. Takagi[4], Y. Nakamura[1].* 1) Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science University of Tokyo Japan; 2) 1st Dept.of Surg., Sappro Med. Univ., Japan; 3) Dept.of Surg., Kurume Univ.,Japan; 4) Laboratory of Genome Database, Human Genome Center, Institute of Medical Science University of Tokyo Japan.

cDNA microarray technology enables us to analyze expression of thousands genes at one time and will revolutionize the method to characterize individual properties of cancer cells. It is obvious that biological properties of cancer cell (potential of invasion and metastasis, sensitivity to chemo-radiotherapy) must reflect expression levels of genes in individual cancer cells. By means of systematic analysis of gene expression profiles, we attempted to examine in detail properties of each cancer and to establish novel diagnostic and therapeutic methods for cancer patients. We prepared microarray containing 4476 genes including ESTs and analyzed the gene expression profile of 60 esophageal cancers. We labeled RNA extracted from cancer tissues of patients with Cye 3 and the other from normal esophageal tissues with Cye 5, and compared gene expressions in cancer and its corresponding normal tissues. By a combination of T7 based RNA amplification method and the microarray system, we examined the relation between gene expression profile and clinicopathological features of each patients (TNM stage, resistance to adjuvant-therapy, prognosis) to find predictive marker genes for malignancy or sensitivity to adjuvant-therapy. Gene expression profile showed various pattern in each patient, but focused on several genes and conbine their expression pattern, we found the correlation between their pattern and some clinical features. We suggest that these results can contribute to the more precise diagnosis of cancer and the selection of optimal therapy to an individual patient.

Program Nr: 711 from the 1999 ASHG Annual Meeting

Biological characteristics of the breast in women with a genetic predisposition to breast cancer. *J.A. Kirk[1], K. Applebee[1,2], J. Leary[1], C. Clarke[2].* 1) Familial Cancer Service, Westmead Hospital, NSW, Australia; 2) Westmead Institute For Cancer Research, University of Sydney, Westmead Hospital, Westmead, NSW, Australia.

Other than the presence of a germline mutation in BRCA1/2/X, there is little known about the subsequent morphological, genetic and hormonal changes that may account for tumour development in the normal breast of a woman genetically predisposed to breast cancer. We have hypothesized that a mutation in a breast cancer susceptibility gene results in an alteration of the biology of the breast tissue of such women. We examined the breast tissue removed at prophylactic mastectomy from women who had previous unilateral breast cancer, and were known or suspected to carry a germline mutation in BRCA1 /2 (test cases, n=12) and compared the findings to those in normal breast (control cohort, n=28) removed for other reasons. We examined the expression of markers of hormone responsiveness (oestrogen receptor [ER], progesterone receptor [PR] and pS2), proliferation (Ki-67, Cyclin D1), tumour suppressor genes (p53, BRCA1) and molecules often overexpressed in breast cancers (erbB-2), using immunohistochemistry and image analysis. Cyclin D1 and erbB-2 were not detected in test cases or controls. BRCA1, Ki-67 and p53 were low in all samples. ER and pS2 were detected at the same levels in test cases and controls. By contrast, PR expression was significantly lower in the breast tissue of women with a genetic predisposition ($p<0.001$; t-test, assuming unequal variance). BRCA-associated breast tumours are generally less likely than sporadic tumours to express the hormone receptors, ER and PR. We have shown that the expression of PR is reduced in breast tissue of women with a genetic predisposition to breast cancer. PR is a marker of a functional oestrogen pathway in breast cancers, as oestrogen is one of the factors that regulate PR expression in breast tissue. One of the important roles of progesterone and PR may be to inhibit the proliferative action of oestrogen in normal tissue. Loss of PR may provide the opportunity for unopposed oestrogen action, increasing the risk of invasive breast cancer.

Program Nr: 712 from the 1999 ASHG Annual Meeting

***FGF4* and *INT2* oncogenes are amplified and expressed in Kaposi's sarcoma.** *S. Kiuru-Kuhlefelt[1], M. Sarlomo-Rikala[2], M.L. Larramendy[1], M. Söderlund[3], K. Hedman[3], M. Miettinen[4], S. Knuutila[1]*. 1) Department of Medical Genetics, University of Helsinki, Finland; 2) Department of Pathology, University of Helsinki, Finland; 3) Department of Virology, University of Helsinki, Finland; 4) Armed Forces Institute of Pathology, Department of Soft Tissue Pathology, Washington, DC.

Kaposi's sarcoma (KS) is a vascular tumor, the pathogenesis of which has been suggested to include human herpesvirus 8 (HHV-8), as well as various cytokines and growth factors. Very little is known about cytogenetic and molecular genetic changes in KS. We studied DNA copy number changes in KS and found a recurrent gain at 11q13. We then analyzed the amplification and expression status of two known oncogenes, *FGF4* and *INT2* residing at 11q13. Comparative genomic hybridization, interphase fluorescence in situ hybridization with *FGF4* and *INT2* containing YAC probes, and immunoperoxidase immunostaining with anti-FGF4 and -INT2 antibodies were used on twelve KS samples. All samples tested were shown by PCR to be HHV-8 positive. A recurrent gain at 11q13 was shown by CGH in four out of ten cases studied. Out of six cases studied by interphase fluorescence in situ hybridization, four showed a 3- to 4-fold amplification with the *FGF4* and *INT2* containing probes. Expression of *FGF4* and *INT2* was found in nine and three cases, respectively, out of nine studied. Amplification and expression of these genes is particularly interesting in the context of oncovirus involvement, since *INT2* is a homolog of mouse int2, which causes mammary carcinoma in mice when activated by integration of retrovirus MMTV, mouse mammary tumor virus. This rises the question whether HHV-8 represents an integrating oncovirus that causes amplification and activation of genomic oncogenes in man.

Program Nr: 713 from the 1999 ASHG Annual Meeting

Genotype-Phenotype Analysis of Gemistocytic Astrocytomas. *S. Kosel[1], B.W. Scheithauer[2], M.B. Graeber[3].* 1) Institute of Neuropathology, University of Munich, Munich, Germany; 2) Division of Surgical Pathology, Mayo Clinic, Rochester, MN, USA; 3) Department of Neuromorphology, Max-Planck-Institute of Neurobiology, Martinsried, Germany.

Among diffuse astrocytomas, the gemistocytic variant carries the least favorable prognosis. Such tumors are composed mainly of cells with large eosinophilic cell bodies and often eccentric, rather spherical nuclei. We selected 31 examples from among 201 gemistocytic astrocytomas operated at Mayo Clinic between 1985 and 1998. Typical cytologic appearance and relative abundance of gemistocytic cells were the selection criteria. Tumors representing WHO grades 2 (n=8), 3 (n=12) and 4 (n=11) were included in the study. Genomic sequencing of exons 5-8 was performed using an automated ABI377 DNA sequencer. In addition, morphometric analysis of individual tumor cells and tumor cell nuclei was undertaken using the Optimas image analysis program. In 21 cases analyzed for p53 sequence changes, 15 tumors, including 3 grade 2, 7 grade 3 and 5 grade 4 lesions were found to carry mutations. Specifically, 3 mutations were detected in exon 5, 2 in exon 6, 1 in exon 7, and 9 in exon 8 of the p53 gene with 5 patients carrying the C14486T/Arg273Cys variant; 5 mutations were novel. Thus, p53 mutations were present in 71% of the tumors. Patients whose tumors carried a p53 mutation were significantly younger (p=0.002). Furthermore, mutations were present in 7 of 8 anaplastic tumors. Regarding histologic phenotype, the sizes of tumor cell nuclei and of entire tumor cells were positively correlated (p=0.003). There was also a tendency for smaller tumor cell nuclei to be more atypical or less circular (high value of the Optimas circularity function)(p=0.01). Furthermore, there was a weak positive correlation between loss of circularity of tumor cell nuclei and diminished cell size (p=0.045). In summary, our data confirm that the frequency of p53 mutations is significantly higher in gemistocytic astrocytomas as compared to other astrocytoma subtypes. At present it is unclear whether their high frequency of p53 mutations contributes to the more malignant behavior of gemistocytic tumors.

Program Nr: 714 from the 1999 ASHG Annual Meeting

Association between the GSTM1, GSTP1 and GSTT1 gene polymorphisms and young onset prostate cancer in the UK. *Z. Kote-Jarai[1], S. Edwards[1], D. Easton[3], R. Jackson[1], A. Ardern-Jones[2], A. Murkin[2], D. Dearnaley[1,2], CRC/BPG UK Familial Prostate Cancer Collaborators[1], R. Eeles[1,2]*. 1) Cancer Genetics, Institute of Cancer Research, Sutton, UK; 2) Royal Marsden NHS Trust, Sutton, UK; 3) CRC Genetic Epidemiology Unit, Cambridge, UK.

There is an increasing amount of evidence suggesting that polymorphisms in the glutathion S-transferases (GST) are associated with cancer susceptibility and that some of these polymorphisms may have a modifier effect on tumour suppressor genes. The GST supergene family includes several loci with well characterised polymorphisms. Approximately 50% of Caucasian populations are homozygous for deletions in GSTM1 and about 20% are homozygous for deletions in GSTT1, resulting in conjugation deficiency of mutagenic electrophiles to glutathione. Both GSTM1 and GSTT1 null genotypes have been associated with an increased risk of various cancers. The GSTP1 gene has a polymorphism at nucleotide 313 resulting in an Ile to Val substitution which consequently lowers the enzymatic activity of the protein and this has also been suggested as a high risk genotype. We investigated the association between genetic polymorphisms in GST genes and young onset prostate cancer (age of onset £55 years) in a case-control study. GSTM1, GSTT1 and GSTP1 genotypes were determined for 275 young prostate cancer patients and for 280 control subjects by PCR and RFLP. The associations between specific genotypes and the development of prostate cancer were examined by use of logistic regression to calculate odds ratios (OR) and confidence intervals. We found no significant difference between the control group and the prostate cancer cases in the frequency of GSTM1 and GSTT1 genotypes (OR for GSTM1 null 0.86 95%CI 0.62-1.21, for GSTT1 null 0.86 95%CI 0.59-1.26). The GSTP1 genotype had however a significant effect on prostate cancer risk: the Ile/Ile homozygotes had the lowest risk, and there was a trend in risk with the number of Val alleles (Ile/Val OR:1.3 95%CI 0.91-1.87, Val/Val OR:1.75 95%CI 1.03-2.99; $p_{(trend)}$=0.026). These results suggest that the GSTP1 polymorphism is likely to be important in predisposition to young onset prostate cancer.

Program Nr: 715 from the 1999 ASHG Annual Meeting

Detection of BRCA1 and BRCA2 mutations in Saxonian families with a multi-tumor sectrum. *H. Kraus, K. Engelmann, B. Thamm, UG. Froster.* Institute of Human Genetics, University of Leipzig,Germany.

Abstract **Objective:**Alterations in the cancer susceptibility genes BRCA1 and BRCA2 seem to be responsible for most cases of inherited breast and ovarian cancer. Presently, an involvement of these genes in tumorgenesis of other kinds of cancer is unclear. In order to find clues of the putative influence of BRCA1 and BRCA2, breast cancer patients with a family history of breast and/or ovarian cancer, as well as breast cancer patients with a multi- tumor spectrum in the family, were screened for inherited predisposition in the both genes. **Methods:**Nine patients from nine unrelated Saxonian families were investigated to detect germline mutations in the BRCA1 and BRCA2 genes. The cancer spectrum of the members of these families involves leukemia, breast-, bladder-, stomach-, renal-, cervix-, laryngeal-, uterine-, and colon carcinoma. The entire coding regions including exon/intron junctions of BRCA1 and BRCA2 were amplified by PCR. The transcribed exons of BRCA1 were amplified with 28 PCR`s, for BRCA2 41 PCRs were necessary. Both, forward and reverse strands of each PCR product were analysed by direct sequencing using fluorescent dye terminators on an ABI 377 Sequencer. **Results:**Missense and nonsense mutations in both cancer susceptibility genes were detected in four out of nine patients (two BRCA1- associated, and two BRCA2-associated alterations). Three out of four mutation carriers showed a multi-tumor background in the family history. Unclassified variants are not included, but have been found in all patients. **Conclusion:**Alterations in the tumor suppressor genes BRCA1 and BRCA2 seem to play a role also in other cancers than breast and ovarian cancer. The involvement of these genes in tumorgenesis should be clarifed by investigations of tissues of different tumors.

Program Nr: 716 from the 1999 ASHG Annual Meeting

Candidates for the AML tumor suppressor gene mapped to a minimal deletion interval at 5q31. *J. Kravarusic[1], Z.H. Arbieva[1], H. Xie[1], S.K. Horrigan[2], T.T. Le[1], C.A. Westbrook[1].* 1) Section of Hematology/Oncology,Department of Medicine, Univ.Illinois at Chicago, Chicago, IL; 2) Department of Pediatrics, Lombardi Cancer Center, Georgetown University Medical Center, Washington, DC.

Interstitial deletions of chromosome 5 are frequent in malignant myeloid disorders such as acute myeloid leukemia, and are associated with a poor prognosis. The consistent occurrence of deletions at 5q31 suggests that it is the site of a tumor suppressor gene (TSG). We have attempted to further narrow this interval and identify genes within it as candidates for the TSG. To specify a narrower interval, we selected polymorphic markers from a high resolution physical, genetic, and radiation hybrid (RH) breakpoint map of 5q31, and used them to allelotype a case of AML with a sub-cytogenetic del(5q), comparing DNA from leukemic cells to buccal mucosa from the same patient. Loss of heterozygosity was observed for markers D5S476 and D5S1372, with retention of flanking markers D5S500 to D5S594, thus specifying a deletion interval of about 700 kb in size. The resolution of the RH breakpoint map of the interval was improved by additional typing of selected hybrids, and was then used to identify genes and ESTs from the Radiation Hybrid Database (Rhdb). As a result of the study, we have localized to the target interval four unknown ESTs and three known genes, including HSPA9 (a cellular immortalization protein or mortalin), CTNNA1 (alpha catenin), and EGR1. Additional ESTs in flanking RH bins are also under consideration. Appropriate expression of the candidate sequences in CD34+ cells and leukemic cell lines has been confirmed, and assembly of full-length cDNA and mutational evaluation in AML is underway.

Program Nr: 717 from the 1999 ASHG Annual Meeting

PTEN transcription in mouse development. *H. Kremer[1], M. Nelen[2], J. Schepens[3], W. Hendriks[3], G. Padberg[2]*. 1) Human Genetics, Univ Hospital, Nijmegen, Netherlands; 2) Neurology, Univ Hospital, Nijmegen, Netherlands; 3) Cell Biol and Histol, Univ Nijmegen, Nijmegen, Netherlands.

The PTEN gene is one of the most commonly mutated tumor suppressor genes in human cancer.The PTEN protein is a phosphatase and influences the activity of PI-3-kinase signaling pathways by regulating the levels of PIP3. Germline mutations in the gene can cause Cowden disease (CD). Characteristic for CD are hamartomatous features of several tissues and an increased risk to develop multiple neoplasias. The presence of developmental defects in CD, mainly macrocephaly, suggests a role of PTEN in development which is underlined by the death of PTEN-knockout mice before E10.

To provide more insight into the function of PTEN in development we have performed RNA in situ hybridization using the PTEN coding region as a probe on cryosections of mouse embryos and on brain sections of stages in postnatal development. The PTEN gene is ubiquitously transcribed. In stage E12.5 the highest transcript level is seen in the dorsal root ganglia and high levels are seen in the developing liver, thymus and in the primordia of the follicles of the vibrissae. Also several epithelia e.g. of the tongue and the stomach show an elevated level of transtripts. Transcription of PTEN in brain is homogeneous with the highest levels in the forebrain. At the stages E14.5 and E16.5 the above mentioned high transcript levels are even more prominent. Remarkably, several of the mentioned tissues are symptomatic in CD. During postnatal brain development both in the cerebral cortex and the cerebellum a general PTEN transcription changes into a more differentiated pattern and into a general expression again at postnatal day 20. The highest expression of PTEN in brain is seen in the hippocampus from postnatal day 4 up to adulthood.

Our results do not give evidence for a specific function of PTEN in development either in growth, differentiation or developmental apoptosis but suggest a versatile role of PTEN throughout development.

Program Nr: 718 from the 1999 ASHG Annual Meeting

Molecular But Not Cytogenetic Evidence Of t(11;22)(q24;q12) Translocation In A Patient With Ewing's Sarcoma(EWS)/Primitive Neuroectodermal Tumor(PNET): Immunohistochemical, Histological, Cytogenetic, And Molecular Studies And A Review Of The Literature. *M. Krishna[1], G.K. Turi[1], M. Ladanyi[2], J.J. Ding[1], D.M. Iancu[1], G. Nuggehalli[1], D.P. Bartok[1], V.C. Angadi[1], C.B. Cunha[1]*. 1) Department Of Pathology, Cytogenetics Laboratory, Winthrop-University Hospital, Mineola, NY; 2) Department Of Pathology, MEM Sloan-Kettering Cancer Center, New York, NY.

The t(11;22)(q24;q12) translocation is a characteristic chromosomal marker of Ewing's sarcoma(EWS) and is important in the differential diagnosis of EWS with respect to other mixed round cell tumors, such as rhabdomyosarcomas or lymphomas. Accurate diagnosis is therefore essential for prognosis and clinical management of the EWS disease. Here we describe a clinical case in which the cytogenetic analysis of extraskeletal tumor of a 40-year-old male, diagnosed as EWS on the basis of immunohistochemical and histologic profile, revealed a 45,X,-Y,t(2;4)(q37.1;q13.2), t(14;21) (q22;q21.2)[28]/46,idem,+8[2] karyotype with no evidence of the t(11;22) translocation. In view of this unexpected cytogenetically negative result, molecular studies, using reverse transcriptase-polymerase chain reaction (RT-PCR) test, were performed, and the results yielded EWS/FLi1 fusion transcripts, demonstrating the presence of t(11;22) rearrangement at the molecular level in the tumor. From the clinical point, this case demonstrates the importance of using more than one diagnostic modality to reach an accurate diagnosis. If the diagnosis was based on cytogenetic results alone, the case would have been false-negative, and would have resulted in improper clinical management of the disease. The results of this study will be reviewed along with other similar cases reported in the literature.

Program Nr: 719 from the 1999 ASHG Annual Meeting

Infrequent *MSH2* and *MLH1* gene carriers among newly diagnosed colon cancer patients of Eastern Ontario.

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Mutations in *MSH2* and *MLH1* account for 50% of the families meeting the Amsterdam criteria for HNPCC, and for ~10% of the families that do not. Individuals considered at risk of being predisposed in the non-familial group include ~30% of patients younger than 30-35 yr of age, and 10% of patients having tumors expressing overt microsatellite instability (MSI). However, most common cancers and an appreciable proportion of HNPCC cases are diagnosed beyond the age of 40; the predictive values of the MIS markers are modest. This sudy was undertaken to provide an appreciation of the incidence of *MSH2* and *MLH1* mutations in a series of n= 72 newly diagnosed but otherwise unselected colon cancer patients.

Collateral MIS/familial/clinical and pathological information was available. The mutational analysis of tumor/mucosal DNAs investigated the entire coding regions, 70% by SSCP and 30% by direct sequencing. A total of four germline mutations were characterized, giving an uncorrected frequency of 5.6% (*95%CI*= 1.6-13%). The causality of two missense (Ser269Thr, Val326Ala) and one intronic (exon 11, -11 T/A) *MLH1* mutation could not be established, leading to a more conservative estimate of 1.4% (*95% CI*= .04-7%). The single pathogenic mutation found out of 72 tested cases was the recurrent G(exon5, +3nt)A donor splice *MSH2* mutation, present in an MIS-positive and HNPCC patient. Several polymorphisms but no mutations were identified in other age, familial colorectal, familial extracolonic, sporadic or MSI stratified subgroups. These findings corroborate the Finnish study by Aaltonen et al. and suggest that the *de novo* rate of *MSH2* and *MLH1* mutation contributes minimally to the burden of colon cancer. Thus, predictive testing targeting the colon cancer population has limited advantage over the prior ascertainment of cancer-prone families through genetic clinics and registries, and the inclusion of other genetic loci (*MSH6, TGFBR2* in the molecular assessment.

Program Nr: 720 from the 1999 ASHG Annual Meeting

Founder mutations in BRCA1 in the North West of England. *F. Lalloo[1], E. Redmond[1], C-L. Wu[1], D. Bourne[2], F. Macdonald[2], E. Maher[2], DGR. Evans[1]*. 1) University Department of Medical Genetics and Regional Genetics Service, St Mary's Hospital, Manchester, UK; 2) Regional Genetics Service, Birmingham Womans Hospital, Birmingham, UK.

Mutations within BRCA1 are spread throughout the coding exons of the gene. Within certain populations, founder mutations occur with increasing frequency. For example, two mutations, 185delAG, and 5382insC account for the majority of the mutations in BRCA1 amongst the Ashkenazim. Within the UK a strong founder effect has not been reported. The population covered by the Clinical Genetic departments of Manchester and Birmingham covers the North West of England and consists of 9.7 million. To date, 389 samples from affected women have been screened for mutations in all or part of BRCA1. A total of 80 mutations have been detected of which 11 have been detected more than once. The Jewish mutations account for two of these and have only been found in families with a Jewish background. The remaining 9 mutations account for 27 of the other 66 mutations detected. In particular, the 4182delAATC mutation has been detected in 5 samples. The pedigrees suggest that these families are unrelated. I will present data on the haplotype analysis of these recurrent mutations. It may be possible to target BRCA1 screening in the North West initially to a small number of specific mutations.

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Program Nr: 721 from the 1999 ASHG Annual Meeting

Analysis of PAX-FKHR fusion positive vs. fusion negative alveolar rhabdomyosarcomas reveals distinct chromosomal patterns and novel genomic changes. *V.S. Lestou[1,2], E.R. Lawlor[1,2], P.H.B. Sorensen[1,2].* 1) Dept Pathology Lab Med, Univ BC, Vancouver, BC, Canada; 2) BCs Childrens Hospital, Vancouver, BC, Canada.

In this study, 17 alveolar rhabdomyosarcoma (ARMS) cases were analyzed for PAX3- or PAX7-FKHR gene fusion status by RT-PCR. Eight PAX3-FKHR fusion positive and nine PAX3-FKHR fusion negative ARMS cases were identified. There were no PAX7-FKHR cases identified. To determine whether there are specific patterns of genomic alterations that can distinguish between PAX3-FKHR fusion positive vs. negative ARMS cases, comparative genomic hybridization (CGH) was performed on primary tumor tissue. Fusion positive ARMS demonstrated prominent chromosomal gains involving 1q (n=5), 2 (n=2), 2p23-p24 (n=3), 2q34-q35 (n=5), 12 or 12q12-14 (n=6), 13q13-qter (n=5), 14q12-q21 (n=3), and 17q22-qter (n=3), and prominent deletions of 1p34-p36 (n=4) and 13q21-qter (n=3). Gain of 13q13-qter (n=5) was always associated with gain of 2 or 2q34-q35. Fusion negative ARMS demonstrated prominent chromosomal gains of 2 (n=4), 8 (n=4), 11p (n=3), 17q22-qter (n=3), and 20 (n=4), and deletions involving 1p34-p36 (n=4), 9p (n=5), 11 (n=4) and 15q21-qter (n=4). Interestingly, two cases of fusion negative ARMS demonstrated genomic changes typical of fusion positive ARMS, i.e. gain of 2q35 associated with deletion of 13q21-qter and/or amplifications 2p23-24 and 17q22-qter. One of these cases showed a cryptic t(1;13)(p36;q14) PAX7-FKHR fusion which was detectable by FISH but not by RT-PCR. Moreover, the clinical features of this case are highly suggestive of a PAX7-FKHR gene fusion. The second case and the molecular characteristics of both cases are currently under evaluation. We suspect such cryptic PAX-FKHR fusions may represent a subset of fusion negative ARMS cases. In summary, a comparison of fusion positive vs. negative ARMS revealed common genomic gains involving parts or whole chromosomes 2 and 12 as well as deletions of 1p34-36. In contrast, amplification of chromosome 8 and losses or gains of 11 was found only in fusion negative ARMS, whereas gain of 2q34-q35, gain of 13q13-qter, and gain of 17q22-qter were restricted to fusion positive or cryptic fusion positive ARMS.

Program Nr: 722 from the 1999 ASHG Annual Meeting

Molecular Cytogenetic Analysis of Uterine Leiomyoma And Leiomyosarcoma by Comparative Genomic Hybridization. *B. Levy, T. Mukherjee, K. Hirschhorn.* Mt Sinai School of Medicine,New York, NY.

Uterine leiomyomata (ULM) are benign smooth muscle neoplasms associated with abnormal uterine bleeding, infertility and abdominal pain. They are also the leading indication for approximately 30% of all hysterectomies performed in the United States. ULM comprise the most common uterine tumors and occur in about 25-30% of women of reproductive age. Uterine leiomyosarcomata (LMS) are presumed to be the malignant counterpart to ULM with less than 1% of ULM cases progressing to ULMS. Transformation of ULM to ULMS is yet to be conclusively confirmed and it may well be that ULMS arise through independent events. Traditional cytogenetic studies have not demonstrated any characteristic chromosomal aberrations common to all ULM and ULMS. Comparative genomic hybridization (CGH) 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 12 ULM and 5 ULMS. CGH analysis of ULM demonstrated chromosomal imbalances in 8 of 12 (66.7%) specimens. Of the 8 ULM specimens that showed DNA copy number changes, all displayed an overrepresentaion of chromosomal material at 9q34, a novel finding. Seven of the 8 abnormal specimens showed gains on chromosome 19 with the minimal region of overlap mapped to 19p13.3 (in 5 cases) and 19q13.1-q13.2 (5 cases). Three of these 7 cases showed gains in both the long and short arm of chromosome 19. Other chromosomal anomalies observed in ULM specimens included gains and losses of chromosome 1p, losses on 7q and gains on 12q. All ULMS specimens demonstrated chromosomal aberrations. Chromosome 1 imbalances primarily included gains on the long arm and losses on the short arm. A loss of chromosome 14 long arm material was observed in three of five ULMS samples. Gains, particularly on the long arm, were concomitantly observed on chromosomes 8 and 12 in 3 of 5 cases. Although overlapping anomalies were observed in some cases of ULM and ULMS, no specific anomalies were evident for all ULM and ULMS. The absence of specific anomalies common to all ULM and ULMS therefore argues against them being benign/malignant counterparts.

Program Nr: 723 from the 1999 ASHG Annual Meeting

Differential ovarian cancer survival in Ashkenazi BRCA1/BRCA2 carriers. *E. Levy-Lahad[1], B. Kaufman[2], S. Eisenberg[1], O. Gottfeld[1], U. Beller[3], P. Renbaum[1], A. Lahad[4], R. Catane[2].* 1) Medical Genetics Unit; 2) Istitute of Oncology; 3) Division of Gynecologic Oncology, Shaare Zedek Medical Center; 4) Dept. of Family Medicine, Hebrew University Medical School, Jerusalem, Israel.

A high precentage of ovarian cancer (ovca) in Ashkenazi Jews (AJ) (~30%) is associated with founder BRCA1/2 mutations. To further characterize BRCA1/2-associated ovca we studied a group of 52 AJ women with epithelial ovca seen sequentially at our Gynecologic Oncology service. 32 of these women were consecutively diagnosed after 1/1994. All patients answered an epidemiological questionnaire and medical records were reviewed. Genomic DNA samples were tested for the AJ founder mutations (BRCA1- 185delAG and 5382insC, BRCA2-6174delT). Of the 52 women 28(54%) were non-carriers (NC), 15 (24%) were BRCA1 carriers and 9 (17%) were BRCA2 carriers. Of consecutively diagnosed women 8 (25%) were BRCA1 and 4 (12%) BRCA2 carriers. Mean age at diagnosis was significantly younger in BRCA1 carriers (50.5$\pm$9.1) compared to NC (58.7$\pm$11.2, p=0.02) and significantly older in BRCA2 carriers (65.3$\pm$7.9) compared to BRCA1 carriers (p=0.001). There were no significant differences in pathology or stage at diagnosis: 4 stage IV (3 NC, 1 carrier), 39 (21 NC and 18 carriers) stage III, and 9 stage I (4 NC, 5 carriers). Survival was analyzed by Cox regression controlled for age-at-diagnosis. BRCA2 carriers had significantly better survival than NC, (all cases: HR 0.14, p=0.012, consecutive only: HR 0.15, p=0.08). Median survival in consecutive cases was 35$\pm$13.4 mos in NC and 52$\pm$23 mos.in BRCA1 carriers. In BRCA2 carriers the median was not reached. Our results suggest that BRCA2-related ovca in AJ is more benign, with an older age-at-diagnosis and improved survival. Older age at diagnosis is consistent with lower penetrance of BRCA2 vs. BRCA1 in AJ. Improved survival was found even though older age at diagnosis is generally an adverse prognostic feature in ovca. Older age at diagnosis and improved survival could be a feature of BRCA2-related ovca, or represent an allelic effect of the 6174delT mutation. Differential ovca survival in BRCA1 and BRCA2 carriers may have confounded previous survival analyses in the AJ population.

Program Nr: 724 from the 1999 ASHG Annual Meeting

Genomic characterization of a region commonly deleted in sporadic breast cancer. *T.B. Lewis[1], C. Wu[2], G. Gloeckner[3], P. deJong[2], A. Rosenthal[3], J. Tischfield[4].* 1) Department of Human Genetics, University of Utah, Salt Lake City, UT; 2) Roswell Park Cancer Institute, Buffalo, NY; 3) Department of Genome Analysis, Institute for Molecular Biotechnology, Jena Germany; 4) Department of Genetics, Rutgers University, Piscataway, NJ.

The occurrence of nonrandom allele loss in sporadic cancer implies the functional inactivation of a tumor suppressor gene at, or near, the tested loci. One of the most common aberrations in sporadic breast cancer involves the long arm of chromosome 16. Deletion mapping of chromosome 16q has suggested 2 distinct regions harboring tumor suppressor genes involved in breast cancer, 16q22.1 and 16q24.3. Loss of these regions has been reported to occur in 23-67% of the cases examined.

A deletion map of the chromosome 16q24.3 region was derived by typing 16 polymorphic markers through 57 sets of DNA from sporadic breast tumors. Loss of heterozygosity was observed for at least one chromosome 16q24.3 marker in 36.8% (21/59) of our patient samples. Analysis of the loss of heterozygosity results allowed identification of the critical region of deletion as between markers D16S3028 and D16S3048, a distance of less than one centimorgan based on genetic maps.

We have assembled and sequenced a PAC contig spanning the region identified by markers D16S3028 and D16S3048. Through exon trapping we isolated 17 clones, direct selection provided 9 clones, and database searches identified 11 Unigene collections. These ESTs merge into 13 separate transcript groupings. One of these transcripts is a gene with high homology to the ribosomal L39 gene; 96% similarity and 92% identity for the length of the L39 gene plus an additional 24 amino acids. We are currently expanding these transcripts to full-length cDNAs through RACE and library screening. These transcripts are considered positional candidates for the putative tumor suppressor gene involved in sporadic breast cancer.

Program Nr: 725 from the 1999 ASHG Annual Meeting

Fractionated but not single dose X-rays influence mechanisms of *in vivo* loss of heterozygosity. *L. Liang[1], M.S. Mendonca[2], C. Shao[1], L. Deng[1], P.J. Stambrook[3], J.A. Tischfield[1]*. 1) Dept Medical/Molecular Gen, Indiana Univ Sch Medicine, Indianapolis, IN; 2) Dept Radiat Oncol, Indiana Univ sch Medicine, Indianapolis, IN; 3) Dept cell Biol, Neurobiol, and Anatomy, Univ Cincinnati, Cincinnati, OH.

To investigate the effect of radiation on *in vivo* LOH, B6C3F1 mice, which were heterozygous at *Aprt* (adenine phosphoribosyltransferase), were whole-body irradiated with single doses of 1, 2, or 4 Gy, or 1 Gy/week X 4 weeks doses of 250 Kvp X-rays. APRT-deficient T cells, a consequence of *in vivo* LOH, were selected in medium containing 2,6-diaminopurine (DAP), an adenine analog that is toxic only to cells with APRT enzyme activity. No significant increase of the median frequency of DAP-resistant (DAP^r) T cell variants was observed in mice exposed to either single doses of 1, or 2 Gy, or fractionated doses of irradiation. Only the 4 Gy single dose of X-rays increased the median frequency of DAP^r variants (~2 fold). Allele-specific PCR of *Aprt* demonstrated that 60-70% of DAP^r variants lost the $Aprt^+$ allele in both unirradiated and irradiated mice. The DAP^r variants exhibiting loss of $Aprt^+$ were genotyped with chromosome 8 linked polymorphic microsatellite repeat markers. In the unirradiated mice and the mice exposed to single dose of X-rays, 95% of the variants exhibiting loss of $Aprt^+$ showed LOH at the most telomeric marker (73 cM) and 50% also showed LOH at the most centromeric marker (8 cM), suggesting that mitotic recombination or/and chromosome loss are the predominant mechanisms for generation of these T cell variants. However, in mice exposed to fractionated doses of irradiation, 70% of DAP^r variants exhibiting loss of $Aprt^+$ did not show LOH at any other markers, suggesting interstitial deletion or gene conversion as the predominant mechanisms leading to loss of $Aprt^+$. Our results suggest that the mechanisms causing *in vivo Aprt* LOH in the mouse may differ according to the way in which X-irradiation is delivered.

Program Nr: 726 from the 1999 ASHG Annual Meeting

Evidence of a Founder *BRCA1* Mutation in Scotland. *A. Liede[1], B. Cohen[2], D.M. Black[3], R.H. Davidson[3], A. Renwick[3], E. Hoodfar[1], O.I. Olopade[1], M. Micek[1], V. Anderson[2], R. DeMey[2], A. Fordyce[2], E. Warner[1], J.L. Dann[5], M.-C. King[5], B. Weber[4], S.A. Narod[1], C.M. Steel[2]*. 1) University of Toronto, Toronto, ON, Canada; 2) University of St. Andrews, Scotland, UK; 3) University of Glasgow, Scotland, UK; 4) University of Pennsylvania, Philadelphia, PA, USA; 5) University of Washington, Seattle, WA, USA.

BRCA1 mutations have been identified in breast and ovarian cancer families from diverse ethnic backgrounds. We studied 17 different families with the *BRCA1* 2800delAA mutation; seven were ascertained in Scotland (Dundee, Edinburgh, Glasgow, St. Andrews), five in Canada (Toronto, Victoria) and five in the United States (Chicago, Philadelphia, Seattle). Overall there was a clear preponderance of Scottish ancestry. Genotype analysis performed on key members from 17 families was consistent with a common haplotype, strongly suggesting a single ancestral origin. A possible link was established between two families by tracing their genealogies through the records of the Registrar General for Scotland. This is the first example of a *BRCA1* mutation likely to be derived from a common founder in Scotland. Further studies will be necessary to estimate more accurately the population frequency of the *BRCA1* 2800delAA mutation among unselected cases of breast and ovarian cancer in Scotland and the United Kingdom.

Program Nr: 727 from the 1999 ASHG Annual Meeting

Augment the Incomplete LOH Data Using Expectation-Maximization Algorithm. *X. Liu[1,2], Z. Luo[2], G. Wang[1], Y. Zhao[1,2], C. Wu[1], W. Zhang[1,2], W. Huang[1], Z. Chen[1]*. 1) Chinese National Human Genome Center at Shanghai, Shanghai 201203, China; 2) Institute of Genetics, Fudan University, Shanghai 200433, China.

Scanning LOH (loss of heterozygosity or called allelic imbalance) is a powerful method in cancer genetics of searching candidate tumor suppressor genes. One big weakness of LOH data is the existence of quite large proportion of non-informative data, that is, the homozygotes. Here we suggest a new method to estimate the probability of allelic imbalance for homozygote. The principle is to use the Expectation-Maximization algorithm to recover the homozygote's hidden state given the information of other loci, whose LOH states are associated with the locus studied. The performance of the method is evaluated by applying it to computer simulation data sets. The results showed our method was much more efficient than the simple estimation using the locus' LOH ratio information. With sufficient sample size, our method can typically gave out 90% accuracy for recovering the LOH states of homozygotes of a locus which had five or more associated loci. So it should be very useful for fine-scale LOH mapping, which has quite some associated loci because of the short distance between loci. We also apply our method to the previous published fine LOH mapping data of chromosome 4q in hepatocellular carcinoma. The augmented data showed some narrower candidate regions of tumor suppressor genes. Finally we suggest that a higher accuracy could be achieved if iteratively using association analysis, clustering analysis and our augmentation method.

Program Nr: 728 from the 1999 ASHG Annual Meeting

Cosegregation of familial multiple lipomatosis and hereditary retinoblastoma in a large pedigree. *D.R. Lohmann[1], M. Genuardi[2], M. Klutz[1], M. Freistuehler[1], G. Neri[2], T. Wienker[3], B. Horsthemke[1]*. 1) Dept Humangenetik, Universitatsklinikum Essen, Essen, Germany; 2) Instituto di Genetica Medica, Rome, Italy; 3) Institut fuer Medizinische Statistik, Bonn, Germany.

Predisposition to retinoblastoma (RB, OMIM 180200) is caused by mutations in the RB1-gene. Patients with hereditary RB also have an increased risk for second tumors, notab-ly osteogenic and soft tissue sarcomata. In addition, recent data indicate that the incidence of multiple lipoma (ML, OMIM 151900) is higher in these patients. The majority of predisposing mutations result in premature termination codons. Patients that have inherited such mutant alleles almost invariably develop bilateral RB. However, some rare mutations are associated with a "low penetrance" phenotype mar-ked by reduced expressivity (unilateral RB) and incomplete penetrance. Up to now there are no data indicating that distinct RB1 mutations are associated with an increased risk for specific second tumors. Here we report on a 4 generation pedigree with ML and RB. Genotyping of RB1 linked loci showed that all 7 patients with ML, 3 patients with ML and RB, and 2 patients with RB only share the identical haplotype, which is also present in 6 family members without ML or RB. Five of the 6 carriers without ML are under age of 35 years and, therefore, ML may occur later. We also noted a significant segregation distortion in favor of the ML/RB linked haplotype. Mutation analysis of the RB1 gene showed a novel single base substitution in exon 13 that is in phase with the ML/RB phenotype. RT-PCR analysis of RNA from lymphoblastoid cell lines shows that exon 13 is skipped in transcripts from mutant alleles. Although some patients with hereditary RB also develop ML, cosegregation of RB and ML has not been reported before. Within the spectrum of RB1 alleles with low penetrance, the mutation identified here is atypical as it results in loss of a significant part of pocket domain A. At present, we cannot decide if this mutation causes both RB (with low penetrance) and ML (with almost complete penetrance) or if another mutation at a distinct but linked locus causes ML in this family.

Program Nr: 729 from the 1999 ASHG Annual Meeting

An unusual pericentric inversion of chromosome 16 in acute monocytic leukemia [M5b] with primary cutaneous manifestation. *J. Lo Ngeo[1], P. Chandra[1,2], M.A. Bertoni[1], M.J. Macera[1,3], R.S. Verma[1,2,3]*. 1) Wyckoff Heights Medical Center, Brooklyn/New York Hospital-Weill Medical College of Cornell University, New York, NY; 2) SUNY Health Science Center at Brooklyn, NY; 3) Institute of Molecular Biology and Genetics at InterScience, Brooklyn, NY.

An eighty three year old Caucasian male was admitted for dyspnea. Eight months prior to this admission, he was hospitalized for syncope. At that time he had pruritic maculo-papular rash over his body and an enlarged left inguinal node. Hemogram done at this time revealed WBC 2.55 X 10^3/ul, Hb. 14.2g/dl, platelet count 86,000/ul and a normal WBC differential. No blasts were noted in the peripheral blood smear (PBS) on admission. Skin biopsy revealed leukemic blasts infiltrating the skin. He was treated symptomatically as the patient refused chemotherapy. The patient remained well for the next 8 months when he was re-hospitalized for pneumonia. The skin rash got worse. Hemogram revealed WBC 70.9 X 10^3ul Hb.13.2 g/dl and platelets 10.2 X 10^3/ul. The PBS showed 40% blasts. The BM was hypoplastic and islands of promonocytes and monoblasts formed 50% of the cellular elements of BM. The blast cells were positive for muramidase. A clinical diagnosis of acute monocytic leukemia (M5b) was established. Using GTG- and FISH techniques with a probe specific for the 16p arm which detects the 16p13q22 inversion specific for AMMoL(M4) revealed an abnormal karyotype 46,XY .ish inv(16)(p13.3q23) in 50% of his dividing BM cells, while the remaining metaphases were apparently normal. At that time, he was treated with cytarabine and doxorubicin. The skin infiltrates cleared completely and the blasts in the peripheral blood disappeared. Pericentric inversion of chromosome 16 with typical breakpoints(p13q22) have been noted in 2 earlier cases of AML (M5b). However, our case has different breakpoints on the inverted chromosome 16(i.e.p13.3q23). This is of great significance as it may be a bio-marker for indolent disease which is also sensitive to chemotherapy. The inv(16) is shown to be associated with a better prognosis in AML(M4).

Program Nr: 730 from the 1999 ASHG Annual Meeting

Molecular Genetic Profiling of Renal Cell Carcinoma as an Alternative to Current Histopathologic Classification Systems. *S.T. Lott[1], L. Rogers[1], R. Amato[1], L. Wood[1], T. Powdrill[2], D. Thakkar[2], A. El-Naggar[1], A.M. Killary[1]*. 1) MD Anderson Cancer Ctr, Houston, TX; 2) Genometrix, The Woodlands, TX.

The initiation and progression of human cancer is a multistep process involving genetic alterations of critical genes controlling the destiny of defined cell lineages with regard to cellular proliferation, differentiation or death. Elucidation of the genetic pathways underlying these growth control processes is crucial to our understanding of the origins of the malignant state. Furthermore, definition of genetic pathways intimately involved in particular cancer types should lead to the improved clinical treatment of cancer. Our laboratory has undertaken an extensive genetic, molecular biologic, pathologic, and statistical approach to generate a comprehensive molecular profile of renal cell carcinoma (RCC). Currently, classification of RCC tumors is largely based on histopathological parameters, which do not directly correlate with clinical outcome. Our approach has centered on the use of a commercially developed microarray consisting of oligonucleotides corresponding to 44 genes frequently involved in the initiation and development of human tumors (Genometrix, Inc., The Woodlands, TX). Pathways represented on this array include cell signaling, apoptosis, cell cycle control and drug resistance. To explore the involvement of these genes in the development of RCC, we made use of a panel of twenty RCC matched tumor/normal samples obtained from patients undergoing nephrectomy at the M. D. Anderson Cancer Center. Fluorescently labeled, reverse transcribed cDNA from matched tumor/normal samples were simultaneously hybridized to the array. Following posthybridization washes, arrays were scanned to determine the amounts of fluorescently labeled probe bound at each element of the array. Relative expression of each gene was determined by comparing the average intensity of the hybridized material at each element to that of b-actin and GAPDH. Together, these studies have greatly expanded our understanding of the basic genetic changes associated with renal tumorigenesis.

Program Nr: 731 from the 1999 ASHG Annual Meeting

Chromosomal clues to the development of metastatic prostate neoplasm. *C.A. Luhrs[1], G. Morel[1], M.J. Macera[2,3], R.S. Verma[2,3]*. 1) Brooklyn V.A. Medical Center, Brooklyn, NY; 2) Institute of Molecular Biology and Genetics, Brooklyn, NY; 3) Wyckoff Heights Medical Center, Brooklyn, NY/New York Hospital-Weill Medical College of Cornell University, New York, NY.

A 65 year old Caucasian male was diagnosed with metastatic carcinoma of the prostate on December 1997. He had been recently evaluated for increasing fatigue, peripheral lymphadenopathy, splenomegaly, increased LDH (2625) and worsening anemia. A peripheral smear showed leucocytosis with predominant immature lymphoid cells. (CD20+). Flow cytomerry showed B cell lymphoma and abdominal CT revealed bulky mesenteric LDP and retroperitoneal, pelvic lymph nodes. Enlarged spleen with peripheral infarcts was observed. Cytogenetic evaluation of bone marrow cultured for 48 hours was performed by GTG- and FISH- techniques. A total of 50 metaphases were analyzed and highly complex chromosomal abnormalities of varied nature were characterized by the FISH technique. Cytogenetic evaluation of bone marrow in patients with metastatic prostate cancer has been very rare. No common chromosomal abberations were found by comparing with earlier cases. The chromosomal abnoromalities found in those cases could be random, representing secondary events or caused by chromosomal instability due to the culture conditions of cancer cells. However, Li and associates [Li et al Genes Chromosomes & Cancer 24:175-182, 1999] suggested that 16q23-24 could be a frequent aberration in patients with matastasis of prostate cancer.

Program Nr: 732 from the 1999 ASHG Annual Meeting

Transcriptional Regulation of Cyclooxygenase-2 Gene Expression : Novel Effects of Non-Steroidal Antiinflammatory Drugs. *A. Mandal, C-J. Yuan, Z. Zhang, A. Mukherjee.* SDG/HDB, NICHD/NIH, Bethesda, MD.

The cyclooxygenase-2 (COX-2) gene overexpression is suggested to play important roles in colorectal tumorigenesis. Epidemiological studies revealed that non-steroidal antiinflammatory drugs (NSAIDs), such as aspirin and sulindac that inhibit COX activity, reduce colorectal cancer mortality. Current investigations have focused on delineating the molecular mechanisms that regulate the COX-2 gene expression and the roles of NSAIDs in cancer chemoprevention. COX-2 catalyzes the production of prostaglandins (PGs) from arachidonic acid (AA), generated by phospholipases A2 (PLA2s), a family of acyl esterases that cause the release of AA from cellular phospholipids. Pancreatic secretory PLA2 (sPLA2), via its receptor (sPLA2R), transcriptionally activates the COX-2 gene expression in several cell types, although a specific transcription factor mediating COX-2 expression has not yet been identified. Here, we report that transcription factor, C/EBPb, plays a critical role in sPLA2IB-induced, receptor-mediated, COX-2 gene expression in MC3T3E1 cells. Furthermore, treatment of these cells with NSAIDs stimulates COX-2 mRNA and COX-2 protein levels with concomitant elevation in PG production. Most importantly, NSAID treatment of the cells drastically suppresses the production of cytosolic PLA2 (cPLA2)-mRNA. The lack of sPLA2IB-, sPLA2IIA- and sPLA2V-mRNA expression in both NIH3T3 and MC3T3E1 cells suggests that cPLA2 is the most likely enzyme that catalyzes the release of AA, the rate-limiting substrate of COX for the production of PGs. Our results, in addition to defining a critical role of C/EBPb in the regulation of COX-2 gene expression, demonstrate that despite the apparent stimulation of COX-2-mRNA expression, NSAIDs may inhibit PG production by drastically suppressing cPLA2-mRNA. Since both AA and PGs regulate many biological functions that are characteristic of most cancer cells and have profound effects on cellular differentiation, we propose that the previously reported anti-metastatic and cancer chemopreventative effects of NSAIDs stem from their combined inhibitory effects on cPLA2-mRNA expression, and on COX-2 catalysis.

Program Nr: 733 from the 1999 ASHG Annual Meeting

Phenotype of CDKN2A Arg24Pro carriers and their non-carrier relatives in a large Australian melanoma kindred. *G. Mann[1], C. Ang[2], L. Fritschi[3], E. Holland[1], H. Schmid[1], R. Kefford[1], J. Kelly[2].* 1) Westmead Institute for Cancer Research, NSW 2145, Australia; 2) Victorian Melanoma Service, Alfred Hospital, Vic 3181, Australia; 3) Dept Epidemiology & Preventive Medicine, Monash University, Vic 3168, Australia.

Germline mutations in the CDKN2A gene are observed in some cutaneous melanoma kindreds. Such kindreds are typically ascertained by the presence of multiple cases; affected family members might therefore share risk factors other than the mutant CDKN2A allele. We have studied 64 individuals in the fourth and fifth generations of a familial melanoma kindred residing in Victoria, Australia (lat. 38°S), in which 15 carriers of the Arg24Pro mutation, but no non-carriers, have developed melanoma. The number, size and distribution of melanocytic nevi and lentigines, and a variety of other melanoma risk factors was determined in 21 carriers, 24 non-carrier relatives descended from the same founders, and 19 spouses.

All nevus metrics (mean total nevus number [ANOVA $p=0.003$], mean total nevus density [$p<0.003$], and mean number of dysplastic nevi [$p=0.001$]) were highest in mutation carriers (695, 761, 13.5 respectively), lowest in spouses (302, 194, 0.8), and at intermediate levels in non-carrier relatives (422, 329, 3.5). The Arg24Pro carrier state therefore causes increased formation of both common and dysplastic nevi. Differences between non-carrier relatives and spouses, though not statistically significant, suggested that family members share risk factors for nevus formation other than the Arg24Pro mutant allele. Mutation carriers also reported more non-melanoma skin cancers ($p=0.02$) and severe sunburns ($p=0.02$) than non-carriers and spouses, and exhibited the highest numbers of shoulder lentigines ($p=0.02$), a correlate of prior sunburns. Germline Arg24Pro mutation of CDKN2A, which affects p16INK4A but not p14ARF, might therefore also confer increased sensitivity to solar UV radiation.

Program Nr: 734 from the 1999 ASHG Annual Meeting

Fluorescent in situ hybridization study of c-myc oncogene copy number in prostate cancer. *H.F.L. Mark[1,2,4], M. Samy[3], K. Santoro[4], A. Ashraf[2], S. Mark[1], D. Feldman[5]*. 1) KRAM Corporation, Barrington, RI; 2) Brown University School of Medicine, Providence, RI; 3) Women & Infants Hospital, Providence, RI; 4) Rhode Island Hospital, Providence, RI; 5) Yale University School of Medicine, New Haven, CT.

We previously conducted a study of 88 cases of prostate cancer in an attempt to identify potential prognostic biomarkers that can distinguish aggressive cases that must be treated immediately. Prostate cancer is a serious disease affecting men worldwide and compromises the quality of life of prostate cancer patients. Biomarkers studied included chromosome 7 trisomy, chromosome 8 trisomy, and HER-2/neu oncogene amplification. These biomarkers were initially studied because trisomy 8 and oncogene amplification of the HER-2/neu gene have been reported in many other cancers, including those studied in this laboratory. In view of the fact that HER-2/neu amplification was not found to play a prominent role in the group of prostate cancer specimens that we studied, an exploration of other biomarkers was felt to be warranted. Thus, we began a pilot study of c-myc oncogene copy number in prostate cancer using the same protocol for fluorescent in situ hybridization and a directly-labeled SpectrumOrange LSI c-myc probe (Vysis, Inc., Downers Grove, IL) on formalin-fixed paraffin-embedded tissue. Out of a total of 36 cases of prostate cancers successfully analyzed thus far, we found 11 (31%) of the tumors exhibiting three or more positive signals for c-myc in 15% or more of the cells. Of these, only 7 tumors (19% of the total cases studied) had 3 or more signals in 20% or more of the cells. No case had 3 or more signals in greater than 25% of the cells. Compared to other molecular probes tested, the c-myc signals were more faint and the quality of the preparation was less optimal than other tumor specimens that we previously studied. Based on the information available thus far, we conclude that an increase in c-myc oncogene copy number was not a prominent finding in our cohort of prostate cancer patients.

Program Nr: 735 from the 1999 ASHG Annual Meeting

LACK OF RECIPROCAL FUSION IN VARIANT PHILADELPHIA CHROMOSOME TRANSLOCATIONS: A Use of Double Fusion Signal FISH and Spectral Karyotyping. *V.D. Markovic[3], D. Bouman[3], J. Bayani[4], S. Kamel-Reid[1,2,3,4], J.A. Squire[1,2,3,4].* 1) Department of Laboratory Medicine and Pathobiology; 2) Department of Medical Biophysics, University of Toronto; 3) Cancer Cytogenetics Laboratory, Toronto General Hospital; 4) Ontario Cancer Institute, Toronto, ON, Canada.

Chromosomal rearrangements other than the classic reciprocal (9;22) translocation are present in about 5-10% of Philadelphia chromosome (Ph) positive Chronic Myeloid Leukemia (CML) patients and are called variant Ph translocations. It is unclear whether they occur subsequent to the classic Ph translocation, or whether they are caused by a single somatic recombinational event. The molecular genetic consequences of Ph producing translocations in CML result in a p210, p190, or occasionally p230 BCR/ABL fusion gene product and the molecular breakpoints in both classic and variant Ph translocations appear to cluster to the same molecular regions. We have used a sequential analysis of G-banding, spectral karyotyping (SKY) and double fusion signal (D) fluorescence in situ hybridization (FISH) on metaphases derived from ten variant Ph translocations to look for the chromosomal location of BCR and ABL genes. Nine patients had a translocation and one had a translocation and an associated insertion. All nine patients with a translocation had the typical Ph chromosome, and had the BCR/ABL fusion gene present on derivative 22. Reciprocal counterparts of ABL and BCR are usually seen as a second fusion signal in the classic Ph translocation. In the subset of CML patients with variant Ph translocations presented here, reciprocal FISH signals were either retained on separate chromosomes, or they were deleted from the karyotype. The patient with an associated insertion had an atypical Ph chromosome, and the fusion FISH signal indicated the BCR/ABL fusion was present on the derivative 9. We believe the BCR/ABL fusion in this case was produced by a chromosomal insertion. This study provides a more comprehensive description of the molecular events associated with chromosomal rearrangements involving the BCR/ABL fusion gene and has implications for cytogenetic laboratories utilizing interphase FISH analysis in CML.

Program Nr: 736 from the 1999 ASHG Annual Meeting

Germline p53 mutations in women with multiple primary cancers. *A-M. Martin[1], B. Amirimani[1], R.M. Howard[1], H.A. Shih[1], T.R. Rebbeck[2,3], B.L. Weber[1,3].* 1) Department of Medicine; 2) Center for Clinical Epidemiology and Biostatistics; 3) Cancer Center, University of Pennsylvania, Philadelphia, PA 19104.

Background: Women with multiple primary cancers, in which one is breast cancer, may be at an increased risk of germline mutations in tumor suppressor genes. In a cohort of women whose germline mutation status for BRCA1 and BRCA2 is negative, mutations in p53 may be identified.

Methods: To address this issue, 173 women were ascertained through a Breast Cancer Risk Evaluation Program. Eighty-nine women with breast cancer and at least one other primary cancer were screened along with 84 control women who had breast cancer alone and no family history of multiple primary cancers. There was no significant difference in the number of breast cancers per family. Genomic DNA, from each of the 173 women, was screened for allelic variants in the entire p53 gene using conformation sensitive gel electrophoresis (CSGE). Heteroduplex formation was detected on conformation sensitive gels. In addition, a high throughput screen using a modified, automated fluorescent detection system using the ABI 377 Sequencer^{TM}(Ganguly *et al.,* 1998)was conducted. Mutations were confirmed by direct sequncing.

Results: Eight variants were identified among the cases. Of those, 6 occurred at a frequency of >1% in our cohort, suggesting they may be polymorphisms. The remaining 2 variants may be disease-associated mutations. The frequency of variants in the control set is currently under investigation. The association of variants with the presence of multiple primary cancers will be determined.

Program Nr: 737 from the 1999 ASHG Annual Meeting

Role of Chromosome 3p12-p21 Tumor Suppressor Genes in Clear Cell Renal Cell Carcinoma: Analysis of *VHL*-dependent and *VHL*-independent Pathways of Tumorigenesis. *A. Martinez[1], P. Fullwood[1], K. Kondo[2], T. Kishida[2], M. Yao[2], E.R. Maher[1], F. Latif[1]*. 1) Section of Medical and Molecular Genetics, Department of Paediatrics and Child Health, University of Birmingham, Birmingham, UK; 2) Department of Urology, Yokohama City University School of Medicine, Yokohama, Japan.

Deletions on the short arm of chromosome 3 (3p) are a frequent finding in many common cancers including lung, breast, cervical, ovarian, head and neck, and kidney cancer and it is clear that several tumor suppressor genes (TSGs) map to 3p. Cytogenetic analysis and molecular genetics studies have shown that chromosome 3p deletions and loss of heterozygosity (LOH) for 3p markers are features of Clear Cell Renal Cell Carcinoma (CCRCC) but are rare in non-CCRCC. The von Hippel Lindau (*VHL*) TSG, which maps to 3p25, is a major gatekeeper gene for CCRCC and is inactivated in most sporadic CCRCC. However, we have previously suggested that inactivation of other 3p TSGs may be critical for CCRCC tumorigenesis in CCRCC with (VHL^-) and without (VHL^+) inactivation of the *VHL* TSG. To investigate the role of non-*VHL* TSGs in VHL^- and VHL^+ CCRCC, we analysed 82 CCRCC, of known *VHL* inactivation status, for LOH at polymorphic loci within the candidate critical regions for chromosome 3p TSGs (LCTSGR1 at 3p21.3, LCTSGR2 at 3p12 and at 3p14.2). Chromosome 3p12-p21 LOH was frequent in both VHL^- and VHL^+ CCRCC. However, while the frequency of 3p25 LOH in VHL^- CCRCC was similar to that at 3p12-p21, VHL^+ CCRCC demonstrated significantly less LOH at 3p25 than at 3p12-p21. Although there was LOH evidence for CCRCC TSGs at 3p21, 3p14.2 and 3p12, in both VHL^- and VHL^+ CCRCC, the major CCRCC LOH region mapped to 3p21.3 close to the lung cancer TSG region 1 (LCTSGR1). There was no association between tumor *VHL* status and tumor grade and stage. These findings suggest that *VHL* inactivation alone, is not sufficient to initiate CCRCC and that loss of a gatekeeper 3p21 TSG is a critical event for RCC development in both VHL^- and VHL^+ CCRCC.

Program Nr: 738 from the 1999 ASHG Annual Meeting

Correlation between expression of the matrix metalloprotease-1 (MMP-1) gene in ovarian cancers and an insertion/deletion polymorphism in its promoter region. *M. Matsushima[1], Y. Kanamori[1], T. Minaguchi[1], K. Kobayashi[2], S. Sagae[2], R. Kudo[2], Y. Nakamura[1]*. 1) Human Genome Center, University of Tokyo, Tokyo, Japan; 2) Department of Obstetrics and Gynecology, Sapporo Medical University School of Medicine, Sapporo, Japan.

Matrix metalloproteinases (MMPs), a family of closely related enzymes that degrade the extra-cellular matrix, are likely to be involved in invasion and metastasis of tumor cells. A guanine (G) insertion/deletion polymorphism within the promoter region of MMP-1 influences the transcription of this gene; i.e., the 2G (insertion type) promoter possesses greater transcriptional activity than the 1G (deletion type) promoter. To investigate whether the insertion/deletion polymorphism in the MMP-1 promoter has any correlation with development or progression of ovarian cancer, we compared the frequency of each allele in DNAs from 163 ovarian-cancer patients with control samples from 150 healthy women. The frequency of heterozygotes and homozygotes for the 2G allele was significantly higher among women with ovarian cancers (89%) than in individuals without cancer (80%; $p = 0.028$), indicating that individuals who carry even one 2G allele are more susceptible to ovarian cancer than women with a constitutional 1G/1G genotype. To further investigate whether this insertion/deletion polymorphism influences expression of the MMP-1 gene in ovarian cancers, we performed semi-quantitative RT-PCR experiments using tumor tissues whose genotype had been determined. In tumors carrying 2G alleles (1G/2G heterozygotes or 2G homozygotes), the median expression levels of MMP-1 were more than seven times the median level of tumors having no 2G alleles. The intensities of the MMP-1 bands calculated as a ratio against intensities of corresponding beta-2 microglobulin bands were 1.5 versus 0.2, respectively ($p=0.0038$). Since the 2G type of promoter possesses a binding site for the Ets transcription factor, such binding is likely to lead to higher transcription of MMP-1. An excess of MMP-1 production may contribute to enhance degradation of the extra-cellular matrix, and thereby promote invasion and metastasis of tumor cells.

Program Nr: 739 from the 1999 ASHG Annual Meeting

DNA repair gene polymorphisms and DNA-adduct levels in bladder cancer. *G. Matullo[1], S. Guarrera[1], L. Davico[2], S. Carturan[1], M. Peluso[3], A. Piazza[1], P. Vineis[2].* 1) Dip. di Genetica Biologia e Biochimica, Univ. di Torino; 2) Dip. di Scienze Biomediche e Oncologia Umana, Univ. di Torino; 3) IST, Dipartimento di Oncologia Sperimentale, Univ. di Genova; Italy.

The repair of DNA damage has a key role in protecting the genome of the cell from the insults of cancer-causing agents. Although development of bladder cancer is associated with exposure to tobacco (about 50%) and occupational exposure (about 20%), only a proportion of exposed individuals will develop cancer, suggesting that there is a genetic predisposition. Polymorphisms in several DNA repair genes have been identified; however the impact on repair phenotype has not been elucidated. We analyzed the relationship between the following polymorphisms in two repair genes XRCC1-R399H (exon 10) and XPD-K751G (exon 23) and DNA damage as measured by 32-P DNA-adduct in 123 individuals with bladder cancer and 85 controls. Work is in progress on other genetic polymorphisms (XRCC1-R194W exon 6, XPD-D312N exon 10, and XRCC3-T241M exon 7) of different repair systems. No significant difference has been observed in the genotype distribution between cases and controls for XRCC1-R399H and XPD-K751G, neither stratifying by class of age, or smoking habit, nor considering the possible combined effect of the two genotypes. Significant differences exist between cases and controls for DNA-adduct levels (0.47±0.04 vs 0.20±0.03, $p<0.001$) and smoking (110/123=89.4% vs 47/85=55.3%, $p<0.00001$). Notwithstanding we have not observed a significant difference in genotype distributions and DNA-adduct levels between smokers and non-smokers, even considering separately cases and controls, we have found an interesting higher DNA-adduct level (although not significantly) in individuals with XRCC1-R399H GG-AG vs AA genotypes (0.49±0.05 vs 0.34±0.07, $p=0.15$; maximum values are 2.5 and 0.9, respectively) in smoker cases but not in non-smoker cases. No difference in DNA-adduct levels has been observed among genotypes in smokers and non-smokers controls. These results suggest to further investigate the possible interaction between XRCC1 polymorphisms, smoking and other genetic or not risk factors.

Program Nr: 740 from the 1999 ASHG Annual Meeting

Mosaicism in neurofibromatosis 2. *V.F. Mautner[1], M.E. Baser[2], L. Kluwe[3].* 1) Neurology Department, Klinikum Nord Ochsenzoll, Hamburg, DEU; 2) Los Angeles, USA; 3) Laboratory of Brain Tumor Biology, University of Hamburg, Hamburg, DEU.

It is estimated that 10-15% of neurofibromatosis 2 (NF2) patients are mosaic, but few patients have been reported and the clinical spectrum is poorly characterized. From a large series of adult NF2 patients, we identified 11 mosaic patients by analyzing vestibular schwannomas (VSs), cloned fragments, or leukocytes using heteroduplex analysis. These patients had eight nonsense and three frameshift mutations that occurred throughout the *NF2* coding sequence. The age at onset ranged from 16-39 yrs (median, 21 yrs) and the presenting symptoms were hearing loss (one patient), tinnitus (three patients), skin tumor (two patients), gait disorder (one patient), tongue atrophy (one patient), impaired coordination (one patient), and asymptomatic (two patients). The current age ranged from 29-68 yrs (median, 41 yrs). Eight patients had a severe course and three had a mild course. Seven patients had bilateral VSs and four had unilateral VSs. Ten patients had spinal tumors, seven had intracranial meningiomas, six had skin tumors, six had non-VS cranial nerve tumors, and four had lenticular opacities. When compared to the prevalence of these abnormalities in the classically-defined NF2 patients in this series, the largest difference was in the prevalence of unilateral VSs: 36.4% in mosaic NF2 patients versus 6.2% in classical NF2 patients. These results suggest that the mosaic NF2 phenotype may differ from the classical NF2 phenotype, but require confirmation in a larger number of mosaic patients.

Program Nr: 741 from the 1999 ASHG Annual Meeting

The exon 13 duplication in the *BRCA1* gene is a founder mutation found in geographically diverse populations. *S. Mazoyer, G.M. Lenoir, the Duplication Screening Group.* Laboratoire de Génétique UMR 5641, Faculté de Médecine, Lyon, France.

Most mutation screening methods focus on genomic DNA and, being PCR-based, they do not allow the detection of large DNA rearrangements. Therefore, only 14 large germline insertions or deletions have been described in the *BRCA1* gene as compared to more than 500 point mutations or small insertions and deletions scattered over the whole coding sequence and the splice junctions. Rearrangements, however, were found to represent 25% and 15% of all mutations in 2 independent studies performed on Dutch and American breast/ovarian cancer families respectively.

Recently, we identified a 6 kb duplication comprising exon 13 in the *BRCA1* gene, which creates a frameshift at the mRNA level. It was found in 3 apparently unrelated American families of European ancestry and in 1 Portuguese family. A founder effect was very likely based on haplotype data.

In order to better estimate the frequency and geographic diversity of this duplication, which is difficult to identify if not specifically searched for, we have set up a collaborative screen for this mutation by providing specific primers and a positive control to laboratories from 20 different countries (Australia, Austria, Belgium, Canada, Finland, France, Germany, Great Britain, Hungary, Ireland, Israel, Italy, Netherlands, New Zealand, Norway, Spain, Sweden, Switzerland, Turkey, USA). To date, 37 groups have completed the screening of a total of ~3700 breast and/or ovarian cancer patients (~1/2 Anglo-Saxons) with a family history of either or both cancers (representing the diversity in patients who attend high-risk cancer clinics). Of this set, 13 additional independent patients from Australia (3), Belgium (1), Canada (1), Great Britain (6), New Zealand (1), and USA (1) were found to carry the duplication. Haplotype analyses are being performed to confirm a single origin and to estimate the age of this rearrangement. *(The names and affiliations of all individuals involved in this work will be presented on the poster)..*

Program Nr: 742 from the 1999 ASHG Annual Meeting

Clinical Effects of *WT1* Mutations in a Study of 118 Wilms Tumor Patients. *J.M. McDonald, V. Huff.* Department of Experimental Pediatrics, University of Texas MD Anderson Cancer Center, Houston, TX.

Wilms tumor (WT) is a childhood kidney neoplasm that has a genetically heterogeneous etiology. The one cloned WT gene, *WT1*, located at 11p13, has been reported to be mutated in 6-20% of Wilms tumors. To assess the effect of *WT1* mutations, we compared 44 WT patients whose tumors carried germline or somatic *WT1* mutations with 74 WT patients in whose tumors no *WT1* mutations were detected. The average age at diagnosis for patients with somatic *WT1* mutations and patients with no *WT1* mutations were comparable (44.7 and 42.8 months, respectively). As expected, WT patients with germline *WT1* mutations had an earlier average age at diagnosis (18.3 months) and a higher probability of having bilateral disease (0.591) than the patients with somatic or no *WT1* mutations (0.077 and 0.175, respectively). There was little difference between the two subsets of Wilms tumors in regard to favorable and unfavorable histologies. Anaplasia was noted in one of 32 *WT1* mutant tumors and in three of 43 tumors without *WT1* mutations. Because anaplastic tumor histology does not predict all WT relapses, we also assessed relapse status with respect to *WT1* mutations. There was no statistically significant difference in the frequency of relapse between the patients whose tumors did or did not carry *WT1* mutations. With comparable length of followup for both groups (~48 months), five of 26 (0.192) patients with *WT1* mutant tumors relapsed, while 12 of 51 (0.235) patients whose tumors had no detectable *WT1* mutation relapsed. These data on *WT1* mutations in a large cohort of WT patients suggest that *WT1* mutations do not confer a unique clinical phenotype with respect to age at diagnosis or prognosis.

Program Nr: 743 from the 1999 ASHG Annual Meeting

Cytogenetic and molecular evidence of constitutional mosaic trisomy 8 and hematologic abnormalities in a phenotypically normal woman. *J. Meck, T.J. Chen, L-J. Wong, J. Imholte, K. Perry, N. Qin, S. Baidas.* Georgetown Univ Med Ctr, Wash, DC.

Trisomy 8 has clinical significance as both a constitutional and an acquired abnormality. As a constitutional abnormality the phenotype ranges from normal to severe. Trisomy 8 is also an acquired chromosome abnormality common in myeloid disorders. Individuals with constitutional mosaic trisomy 8 regardless of phenotype are predisposed to develop cancer, most often myelodysplastic syndrome. Our report is significant because genotype analysis was used to show that the trisomy was constitutional in our patient and because she presented with hematologic abnormalities, but not cancer. Our patient is a phenotypically normal 34 year old woman with recurrent oral aphtous ulcers since childhood and increased MCV in the last several years. Bone marrow biopsy was slightly hypocellular with mild megaloblastic changes, but otherwise normal morphologically. Unexpectedly, the bone marrow karyotype revealed trisomy 8 in all 20 metaphases analyzed. A 72 hour PHA stimulated peripheral blood culture had 14/20 trisomy 8 cells; the 24 hour culture had no metaphases. None of the 50 skin fibroblast metaphases were trisomic. Constitutional trisomy 8 mosaicism with the abnormal cells confined to blood/bone marrow would explain the lack of phenotypic abnormalities with the exception of hematologic problems. It is unclear whether her bone marrow findings represent an increased risk for development of myelodysplastic syndrome. To confirm the trisomy as constitutional, we performed genotype analysis using six chromosome 8 genetic markers (heterozygosity 0.73 -0.87). Fluorescent labeled PCR primers were used to amplify microsatellite loci. Allele number and size were determined by GeneScan analysis on an ABI 377. All 6 markers suggested the presence of 3 alleles. Three markers showed 3 different sized alleles, while the other 3 markers showed 2 different sized alleles with a ratio clearly indicative of a trisomy. These results are consistent with our patient having a constitutional trisomy mosaicism resulting from a meiotic error with trisomy rescue. Parental genotyping will be performed for confirmation.

Program Nr: 744 from the 1999 ASHG Annual Meeting

The relationship between blood serotonin and depression in first-degree relatives of individuals with autistic disorder. *R.K. Abramson[1], S.A. Ravan[1], E.H. Cook[2], K.M. Wieduwilt[1], M.L. Cuccaro[1], H.H. Wright[1]*. 1) Dept Neuropsychiatry & Behav, Univ S Carolina Sch Medicine, Wm S Hall Psychiatric Inst, Columbia, SC; 2) University of Chicago School of Medicine.

The purpose of this study was to examine the relationship between whole blood serotonin (5HT) and depression in first degree relatives of children with autistic disorder (AD). Elevated blood serotonin may be inherited. It occurs in 30-40% of individuals with (AD), and is also present in unaffected first and second-degree relatives (Abramson, 1989, Cook, 1992, Kuperman, 1985, Perry, 1991). An increased prevalence of depression has also been reported in first degree relative of individuals with AD (Abramson, 1992, Piven, 1991, Smalley, 1993). In the general population, patients with depression have been found to have significantly lower blood 5HT levels (Banki, 1978, Aberg-Wistedt. 1981). In this pilot study, 77 parents and siblings of children with AD completed the Beck Depression Inventory (BDI) or the Child Depression Inventory (CDI). At the time blood was drawn for 5HT levels, current medication usage was recorded since antidepressant and certain over-the-counter medications affect 5HT levels. A bivariate multiple regression model was used with 5HT as the outcome variable and age, gender, ethnicity and depression score as independent variables in the final model. The overall F-test for the final model was significant ($F=8.83$, $p=0.0001$) and the variables in the model accounted for 59% of the variance in 5HT level. Partial t-tests indicate, after controlling for all other covariates, parents and siblings whose BDI or CDI scores indicated depression had significantly lower 5HT ($t=-2.4$, $p=0.023$). In addition, as previously found, participants who had reported taking serotonin inhibiting medications had significantly lower blood serotonin levels ($t=-4.07$, $p=0.0003$). This study indicates first degree relatives with elevated 5HT may be less likely to meet criteria for depression, and elevated 5HT may be a protective factor in family members of a child with AD.

Program Nr: 745 from the 1999 ASHG Annual Meeting

Familial Robin sequence with Wolff-Parkinson White syndrome. *D.N. Abuelo.* Genetic Counseling Center, Rhode Island Hospital, and Brown University School of Medicine, Providence, RI.

The Robin sequence (micrognathia, cleft soft palate, glossoptosis) can occur in otherwise normal individuals, but frequently occurs as part of multiple malformation syndromes such as Stickler syndrome and trisomy 18. Less commonly, it can be seen in some bony dysplasias and neuromuscular disorders. It has not been reported to occur together with the Wolff-Parkinson-White (WPW) type of cardiac arrhythmia. I have observed a family in which the father had Robin sequence and his two sons were born with both Robin sequence and WPW.

The proband was diagnosed with Robin sequence at birth and had cleft palate repair at 10 months. An episode of supraventricular tachycardia in the newborn period was found to be caused by WPW syndrome. He has downslanting palpebral fissures and joint hyperextensibility. Chromosomes, including FISH for 22q- were normal. Chest X-ray showed no rib gaps. The father had a history of micrognathia and cleft soft palate, which was repaired at age 1, with good catch-up growth of the mandible. He developed scoliosis, which was repaired in his teens. He has very mild downslanting palpebral fissures, but is otherwise not dysmorphic. He does not have facies suggestive of Sticker syndrome and has normal intelligence and a normal EKG. His next son was born with WPW, cleft soft palate, severe micrognathia with glossoptosis and required tracheostomy.

When the Robin sequence occurs without other abnormalities, it is considered to have a low recurrence risk. In this family, there is apparent autosomal dominant inheritance. The WPW syndrome in the siblings could be part of the same unique disorder or could have been inherited separately.

Program Nr: 746 from the 1999 ASHG Annual Meeting

Epilepsy with a particular EEG pattern in one patient with Pitt-Rogers-Danks syndrome. *M.-C. Addor[1], M. Maeder-Ingvar[2], T. Deonna[3], D.F. Schorderet[1]*. 1) Medical Genetics, CHUV, Lausanne, Switzerland; 2) Department of Neurology, CHUV, Lausanne, Switzerland; 3) Department of Pediatric Neurology, CHUV, Lausanne, Switzerland.

Partial deletion of the short arm of chromosome 4 is found in patients with Wolf-Hirschhorn syndrome (WHS). This contiguous gene syndrome is characterized by severe pre- and postnatal growth deficiency, mental retardation, a typical dysmorphic face associated with various malformations and midline defects, and seizures. Pitt-Rogers-Danks syndrome (PRDS) was formerly considered as a different clinical phenotype with intrauterine growth retardation, subsequent dwarfism, unusual facies, mental retardation and epilepsy. FISH and DNA analysis have recently shown that PRDS is caused by a deletion overlapping the critical region of WHS; thus the two syndromes are part of the same clinical spectrum associated with deletion near the terminus of 4p; the phenotypic variation observed is accounted for by allelic variation. A particular electroclinical picture has been reported in a few patients with WHS; this EEG pattern is very similar to the one described in Angelman syndrome.

We report on a clinical, cytogenetic, neurological and electroencephalographical study of a 3-year-old female patient with PRDS. She developped clusters of myoclonic seizures from the age of 18 months, and we could record a clinical-electroencephalographic EEG pattern similar to that described by Sgro et al. in four patients with WHS (Epilepsia 1995, 36 : 1206-1214). Valproate markedly reduced the seizure frequency and had a positive effect on development. As PRDS patients have a less severe phenotype and are recognized later than WHS patients, EEG study could facilitate an earlier diagnosis.

Program Nr: 747 from the 1999 ASHG Annual Meeting

Distinctive facial dysmorphism, abnormal wound healing and global developmental delay. A rare Ehlers-Danlos syndrome variant, or a new syndrome? *L.C. Ades[1,2], D. Little[3], A. Colige[4], J. Chaitow[5]*. 1) Dept of Clinical Genetics, Royal Alexandra Hosp Children, Westmead, NSW , N.S.W., Australia; 2) University of Sydney Dept of Paediatrics and Child Health, Royal Alexandra Hosp Children, Westmead, N.S.W., Australia; 3) Dept of Orthopaedics, Royal Alexandra Hosp Children, Westmead, N.S.W., Australia; 4) Laboratory of Connective Tissue Biology, Liege, Belgium; 5) Visiting Paediatric Rheumatologist, Royal Alexandra Hosp Children, Westmead, N.S.W., Australia.

We report the clinical findings in a 3 year-old girl with distinctive facial dysmorphism, extreme ligamentous laxity and joint hypermobility, abnormal skin fragility, poor wound healing and mild global developmental delay. Her features are similar to the Ehlers-Danlos syndrome (EDS) group of disorders, particularly those of the rare disorder EDS type VIIC (human dermatosparaxis). However, histological study of the skin, and molecular genetic studies do not support this diagnosis. Classically, in human dermatosparaxis, electron microscopy (EM)of reticular collagen in longitudinal sections shows branched, twisted ribbons of banded collagen fibrils and cross sections show a characteristic "hieroglyphic" profile. These findings were absent on EM study of the proband's skin. Contrary to the findings in human dermatosparaxis, procollagen I N-proteinase cDNA sequence was normal in this girl. The synthesis and processing of collagens types I, III and V in skin fibroblasts were also normal. Abnormal skin fragility is demonstrable in decorin knockout mice, and this was considered as another potential candidate gene. However, the decorin cDNA sequence in our patient was normal.

Program Nr: 748 from the 1999 ASHG Annual Meeting

Autosomal dominant inheritance of conductive deafness, external ear malformations and congenital facial palsy: second description. *M.A. Aguirre[1], C. Perandones[1], E.L. Furforo[1,2], M. Perez[1], H.A. Rodriguez[3], L. Alba[1].* 1) Genetica Medica, Centro Nac. Genetica Medica, Buenos Aires, Buenos Aires, Argentina; 2) Genetica Medica, Hosp. Mat. Inf. R Sarda, Buenos Aires, Argentina; 3) Hosp. Pediatr. Prof. J. P. Garrahan, Buenos Aires, Argentina.

In 1983 Sellars and Beighton described for the first time the association of conductive deafness due to stapedial anomalies, external ear malformations and congenital facial palsy, in three siblings and their mother. The transmission of these associated malformations in this family suggested autosomal dominant inheritance. We report on the association of congenital bilateral deafness and congenital unilateral facial palsy in a 2-year-old male child and his mother. The boy was the first child of a young non-consanguineous couple. The pregnancy was uneventful and there was no reported exposure to teratogenic agents. At birth the child was hypotonic and presented umbilical hernia. Physical examination revealed bilateral ear malformations, congenital unilateral facial palsy, abnormal palmar creases and a left undescended testis. Congenital unilateral facial palsy and conductive deafness were also observed in the mother. The karyotype was 46,XY for the child and 46,XX for the mother. To our knowledge the combination of auricular, meatal anomalies plus facial palsy has only been reported once before. The aim of this report is to contribute to the delineation of this rare condition and to provide further evidence for an autosomal dominant pattern of inheritance.

Program Nr: 749 from the 1999 ASHG Annual Meeting

Floating- Harbor syndrome - further characterization of the phenotype. *S. Ala-Mello[1], M. Peippo[2].* 1) Dept Medical Genetics, University of Helsinki, Helsinki, Finland; 2) Dept Medical Genetics, The Family Federation of Helsinki, Helsinki, Finland.

The Floating-Harbor syndrome (F-HS) is a rare syndrome first described in 1973. Since then about twenty cases have been reported. The F-HS is characterised by disturbance in speech development, especially in expressive sppech, characteristic dysmorphic faces and short stature.

We report a second Finnish patient, a 6-year old girl, with the F-HS. Cleft palate was detected at birth. Speech development has been slow, and she spoke single words at 1 year and 8 months of age and sentences of a couple of words at 2 years of age. Her voice has been hypernasal. She walked unaided at 1 year and 10 months of age. Her height has been -2.5SD and at the time of investigation at 6 years of age it was -1.8SD. Her facial features are characteristic to the F-HS, and we represent photographs showing them and give more detailed data. All etiologic studies have been normal.

None of the earlier patients with the F-HS have had cleft palate. Five of them, including our first patient, have been described to have immobile palate, nasal speech, palatal insufficiency or hypernasality. We suggest that cleft palate represents one end of the wide spectrum of palatal insufficiency in the F-HS. Thus, the F-HS can be seen as one of the orofacial clefting syndromes.

Program Nr: 750 from the 1999 ASHG Annual Meeting

Al Aqeel-Sewairi Syndrome: An autosomal recessive syndrome with nodular arthropathy and acrolysis. *A.I. Al-Aqeel, W. Al-Sewairy.* Dept Pediatrics, Riyadh Military Hosp, Riyadh, Saudi Arabia.

Inherited genetic disorders are quite common in Saudi Arabia because of the high degree of consanguinous marriages which accounts for almost 60-80% of all marriages. We describe a new syndrome in a very highly inbred family, with all parents of affected children are first degree cousins, inferring an autosomal recessive mode of inheritance. The syndrome is characterized by mild dysmorphic features with proptosis, maxillary hypoplastic and a small jaw. Distal arthropathy starting in the first few months of life which is progressively increasing and involving more proximal joints with characteristic painful nodules involving palms and soles with bone destruction involving mainly tarsal and carpal bones as well as metacarpal, metarsal and phalanges with generalized osteoporosis. All studies including bone marrow aspirate, nodule biopsy and joint biopsy were negative. In conclusion, we describe a new autosomal recessive syndrome, with dysmorphic features, arthropathy, acrolysis. Molecular genetic studies are in progress to find the causitive gene of this new syndrome.

Program Nr: 751 from the 1999 ASHG Annual Meeting

Carpenter syndrome in eight Arab patients,dominant inheritance suspected. *S.S. Al Arrayed.* Genetic department Salmaniya Medical Ctr, Manama, Bahrain.

Carpenter syndrome is rare autosomal recessive syndrome. The diagnostic criteria are acrocephaly, poly dactyly and syndactyly of fingers and toes. Some patients have congenital heart disease hypogonadism, mild obesity and frequent mental retardation. Thirty-five cases had been reported worldwide, since 1961. We are reporting eight cases with carpenter syndrome, four alive and four diseased from two families. In both families parents are consanguineous. All patients have typical features of carpenter syndrome. All the patients have mild mental retardation. None of the patients have congenital heart disease. The karyotypes in all of them are normal. The father in one family has some of the syndrome features such as, hepertelerism, acrocephaly, epicanthus folds, but without polysyndactyly, and with normal intelligence. Conclusion: In this family the consanguinity supports autosomal recessive inheritance. The fact that the father shows some of the syndrome features may indicate either codominant inheritance or dominant inheritance.

Program Nr: 752 from the 1999 ASHG Annual Meeting

Familial cylindromatosis. *Y. Alembik, C. Stoll.* Serv de Genetique Med, Hopital de Hautepierre, Strasbourg, France.

Familial cylindromatose is an entity characterised by a striking clinical picture of multiple disfiguring tumours located on the face and scalp, which rapidly reccured after excision, had a distinctive histology, and showed familial occurence. A rare subtype of dermal eccrine cylindroma is called turban tumour, a highly descriptive label for the classical total involvement of the scalp with tumours covering the head and causing gross disfigurement. We report a family with dermal eccrine cylindromatosis. A mother 65 years old and her daughter 38 years old were examined. In both the tumors became apparent during late adolescence and initially grow slowly. They continue to develop and grow during life. The lesions are localised on the face and scalp, on the back, the chest, the extremities, the abdomen, the ears and the external genitalia. Individual tumors vary in size from a few millimeters to more than 5 centimeters. The mother had a turban tumour. A sister and a brother of the mother are also affected. A genome search using two families with this disease, provided strong evidence for linkage of cylindromatosis to loci on chromosome 16q12-q13. The gene is likely to be a tumor suppressor gene. This family is under study. Several genes including a retinoblastoma-related gene has been mapped to chromosome 16q12.2.

Program Nr: 753 from the 1999 ASHG Annual Meeting

Severe micrognathia, large ears, atrioventricular, septal defect, symmetrical cutaneous syndactyly of hands and multiple café-au-lait spots: A new syndrome? *N. Al-Sannaa, A.S. Teebi.* Clinical Genetics, Hospital for Sick Children, Toronto, ON, Canada.

An infant of nonconsanguineous phenotypically normal parents presented with unique constellation of craniofacial, cardiac, acral anomalies and multiple café-au-lait spots. Craniofacial manifestations include severe asymmetric micrognathia with strikingly large ears but without maxillary or malar hypoplasia or cleft palate. Cardiac defects include atriventricular septal defect and small patent ductus arteriosus. Acral anomalies are symmetrical and include long fingers and toes with complete cutaneous syndactyly between the third and fourth finger; and second and third toe. Skeletal survey revealed asymmetry of mandible and 11 pairs of ribs. Chromosomal analysis was normal. Psychomotor development in the first 3 months of life was normal. Various acrofacial dysostosis syndromes, mandibuloacral dysplasia, hypomandibular faciocranial dysostosis and the syndrome associating large ears with cleft palate and micrognathia (Say syndrome) were all considered in the differential diagnosis and were excluded. Also, no similar cases have been found in POSSUM and London Dysmorphology databases.

Program Nr: 754 from the 1999 ASHG Annual Meeting

Prenatal ascertainment of a novel Osteosclerotic Skeletal Dysplasia following an abnormal Maternal Serum Analyte Screen. *C.C. Apodaca[1], R.F. Hume[1,3], M.J. Stephan[2], R.K. Wagner[1], B.C. Calhoun[1], L.S. Martin[1,2,3]*. 1) Maternal Fetal Medicine; 2) Developmental Pediatrics; 3) Medical Genetics, Madigan Army Medical Center,Tacoma,WA.

Skeletal dysplasias occur in approximately 1:10,000 live births.Currently lethal skeletal dysplasias can be identified prenatally utilizing advanced sonographic methods.Our patient presented following aberrant maternal serum analyte screening.She is a 28 year old East Timoran who presented at 16 weeks for an ultrasound and genetic counseling after her maternal serum analyte screen results indicated an increased fetal risk of Trisomy 18(MSAFP:0.64 MoM,uE3:0.49 MoM,HCG:0.18 MoM).Pedigree analysis revealed advanced paternal age.Fetal anatomic survey demonstrated slightly bowed femurs. Amniocentesis revealed a normal karyotype,46,XY. Subsequent ultrasounds demonstrated bowing of the femurs, a left femur fracture, and a scalloped contour of the cranial outline.Fetal echocardiography demonstrated mitral and tricuspid valve regurgitation.Neonatal skeletal survey demonstrated basilar skull and cortical thickening of the femurs with no evidence of fractures. Sclerosing bone diseases, e.g.osteopetrosis, pycnodysostosis and dysosteosclerosis were considered in the differential diagnosis.Associated neonatal findings include hypothyroidism and exocrine pancreatic insufficiency.The definitive diagnosis has not yet been elucidated.The pleiotropism of this case is highly unusual.The unique constellation of findings:low maternal serum analytes, cardiac valvular anomalies, fetal fractures progressing to neonatal osteosclerosis, and exocrine and endocrine abnormalities remain to be explained.The association between low maternal serum analytes and the neonatal exocrine and endocrine abnormalities can not be ignored. This case demonstrates that while skeletal dysplasias may be identified prenatally, accurate, definitive diagnosis requires a comprehensive postnatal evaluation, to include clinical exam, radiographic survey, autopsy if indicated and appropriate molecular and biochemical testing. This prenatally identified sclerosing bone disease with multi-organ involvement may represent a new syndrome.

Program Nr: 755 from the 1999 ASHG Annual Meeting

Gorlin syndrome. A rare but recognizable cause of mental retardation. *M. Artigas, E. Ortega, G. Pintos.* Dept Pediatrics, Hosp Germans Trias i Pujol, Badalona, Spain.

Gorlin syndrome is an autosomal dominant condition which comprises mild phenotypic changes, skeletal abnormalities, development of nevoid basal cell carcinomas and, occasionally, other tumors. Mental retardation occurs in 5% of cases. We present a boy who was first seen in the outpatient clinic at thirteen years of age due to mild mental retardation. He was a slow learner with a low level of general language, needing multiple sessions of speech therapy. He developed multiple cavities at an early age for which he underwent several operations.. At present he is following special education. On physical examination he had frontal bossing, broad nasal bridge, prognathism, macrotia, scoliosis, flexion contraction at the elbows and short fifth metacarpal. He also showed three caf au lait spots and multiple nevi over the trunk.. The neurological evaluation was normal. A skeletal survey showed a throraco-lumbar scoliosis, a pseudocystic lytic lesion on the first metacarpal of the right hand and calcification of the falx cerebri. Gorlin syndrome seems more common than previously described and may be difficult to diagnose. The gene responsible for this condition is located on the long arm of chromosome 9, and probably acts as a tumour supressor gene.This needs to be taken into account in the follow up of this patients because they can develop other tumors.

Program Nr: 756 from the 1999 ASHG Annual Meeting

Genetic heterogeneity and therapy potential in hypochondroplasia (HCH): preliminary observations and proposal for an international collaborative study. *A.S. Aylsworth[1], T.E. Kelly[2], S.H. Blanton[2], E. Spector[3], L.A. Fordham[1], R.E. Stevenson[4], J. Sollenberger[3], C.A. Francomano[5], G.A. Bellus[3].* 1) Univ. North Carolina at Chapel Hill; 2) Univ. Virginia, Charlottesville; 3) Univ. Colorado Health Sciences Center, Denver; 4) Greenwood Genetic Center, Greenwood, SC; 5) National Human Genome Research Inst., Bethesda, MD.

BACKGROUND: HCH is characterized by short-limbed dwarfism and typical radiographic changes in the skull, spine, and pelvis that may be mild. Less than 75% of patients with a clinical diagnosis of HCH have a typical fibroblast growth factor receptor 3 (FGFR3) N540K mutation. METHODS: We have begun an international collaborative project to study genotype-phenotype correlation in 300 HCH patients and to measure responses to growth hormone (hGH) treatment in a smaller subgroup. Each patient will have clinical data submitted (measurements, craniofacies, development, etc.), molecular analysis for known FGFR3 mutations, and skeletal radiographs. Radiographic measurements for comparison are being made on patients with achondroplasia (ACH) and controls without skeletal dysplasia. So far, we have molecular and limited clinical data on 142 patients and radiographs from 85 individuals. PRELIMINARY OBSERVATIONS: FGFR3 HCH tends to have more severe short stature, lumbar lordosis, disproportion, macrocephaly, and other craniofacial changes than non-FGFR3 HCH. Developmental delay/learning disability is increased in both groups. Iliac proportions measured from radiographs may be helpful in refining phenotypic definitions; a ratio between two vertical measurements (iliac base/wing height) separates controls (avg=0.48), HCH (avg=0.33), and ACH (avg=0.16); but each group has a broad range and there is overlap. DISCUSSION/PLAN: We will enroll more HCH subjects so that analyses can be performed on data stratified by age and molecular diagnosis, treat a group of 50 children (25 with and 25 without FGFR3 mutations) with hGH to measure growth response, and perform linkage studies using families not linked to FGFR3. Study enrollment information will be available at the meeting for clinical geneticists who follow patients with HCH and wish to participate.

Program Nr: 757 from the 1999 ASHG Annual Meeting

Terminal osseous dysplasia and pigmentary defects: Clinical characterization of a novel X-linked dominant syndrome. *C.A. Bacino[1], R. Amir[1], H. Heilstedt[1], R.A. Sierra[2], R. Lewandowski[2].* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Center for Genetic Services, Corpus Christi, TX.

We describe a four generation pedigree with a total of 10 affected females presenting with limb deformities and pigmentary anomalies over the face and scalp. There were no affected males in the pedigree. Furthermore, some of the affected females had a history of multiples miscarriages, strongly suggesting an X-linked pattern of inheritance with male lethality. There was variability in the clinical expression of the phenotype among the affected individuals, which correlated with skewing of X-inactivation detected using a methylation assay at the androgen receptor locus. The family was initially ascertained through a four month old female with multiple congenital anomalies. She presented with dysmorphic features including hypertelorism, iris colobomas, low set ears, midface hypoplasia, pigmentary abnormalities over the face and scalp and limb anomalies. The limb abnormalities consisted of brachydactyly, more marked on the first and second digits, and widening of the fingertips with bumpy, hard, tumor-like lesions, more prominent on the third and fourth fingers (digital fibromas). The hand creases were markedly abnormal. The toes showed internal deviation of the second through fifth toes and generalized toe hypoplasia. In addition she was diagnosed with an atrial septal defect and a liver hamartoma. The proband's mother, female cousins via the maternal side, maternal aunts, and great grandmother were all similarly affected, some with very severe limb deformities. The bumpy digital lesions regressed with age as no digital fibromas were seen in the adult females. Other physical findings include multiple frenulae, abundant vermillion border of the lower lip, and other skeletal anomalies. This syndrome was previously described in the literature in sporadic cases by Bloem et al. (J Bone Joint Surg, 56-B(4):746-751, 1974) and Horii et al.(Am J Med Gen, 80:1-5, 1998). The family presented here shows a novel X-linked dominant condition with male lethality. Current efforts are underway to characterize the disease at the molecular level.

Program Nr: 758 from the 1999 ASHG Annual Meeting

CLINIC AND GENETIC ANALYSIS OF TWENTY SEVEN CASES OF RECURRENT / FAMILIAL HIDATIDIFORM MOLE. *R. Baez-Reyes, R. García Cavazos.* Dept de Genetica, Instituto Nacional de Perinatologia SSa. Mexico, City.

Hidatiform mole (HM) is a challenge regarding its etiopathology. The incidence of HM in our country ranges from 1:44 to 1:552 pregnancies. The risk of recurrence is about 1%. Twenty seven couples with a background of recurrent HM were received in the Genetics Deparment from 1994 to 1998. Clinical history, pedigree and cytogenetic studies were achieved. Families were dividided into three groups: I) couples with recurrent mole (17) II) couples with recurrent mole whose sisters had the same event (7) III) couples with recurrent mole whose sisters and mothers had the same alteration (3). The analysis of the data suggests and atypical inheritance pattern nontraditional from the mother, since all men of these families did not show any pattern of transmission; the recurrence of HM remained, even with either the programs of attended reproduction. All of the mentioned above has interesting etiological implications upon the cellular behaviour during meiosis in a woman's eggs; howewer, it has been difficult to explain this. Consecuently, we can suggest that our patients followed a pattern of atypical inheritance. Conclusions: Hidatidiform mole has a pattern of genetic inheritance wich follows a familial pattern; this leads us make molecular studies aimed to determine the meiotic origin of the alteration and to figure out the ratio of recurrence in these cases due to risk of long-lasting coriocarcinoma.

Program Nr: 759 from the 1999 ASHG Annual Meeting

Rubinstein-Taybi syndrome: deletions by FISH associated with mispatterning of the visceral left-right axis and death in infancy. *O. Bartsch[1], A. Wagner[1], G.K. Hinkel[1], P. Krebs[2], M. Stumm[2], B. Schmalenberger[3], S. Balci[4], F. Majewski[5]*. 1) Inst of Clinical Genetics, Technical University, Dresden, Germany; 2) Inst of Human Genetics, Otto-von-Guericke-University, Magdeburg, Germany; 3) Practice Dr Sigmund, Passau, Germany; 4) Dept of Pediatrics, Hacettepe University, Ankara, Turkey; 5) Inst of Human Genetics, Heinrich Heine University, Düsseldorf, Germany.

The Rubinstein-Taybi syndrome (RTS, OMIM 180849) is an autosomal dominant disorder of characteristic facial features, broad thumbs, short stature and mental retardation. Previous studies indicated that 4 - 25 % of patients have a chromosomal microdeletion on 16p13.3 of the CBP gene. Using FISH and cosmid probes RT100 (CBP exons 14-31), RT191(exons 3-13) and RT203 (intron 2) we studied 45 RTS patients and found 4 deletions (8.9 %). All deletions were interstitial; 3 spanned the CBP gene (RT100-RT203) and 1 was smaller (RT100 only). Previous studies failed to detect a phenotype-genotype correlation between RTS patients with vs. without a deletion. Our findings suggest a more severe phenotype. Mean age at presentation was .96 yrs vs. 11.12 yrs in patients with vs. without a deletion. Patients A and B with a deletion died in infancy which is rare in RTS and was not observed among the other patients. Patients A and D had accessory spleens, Patient A with hypoplastic left heart, abnormal pulmonary lobulation and agenesis of the right kidney and the right uterine horn. This is the first report of polysplenia and the second report of hypoplastic left heart in RTS. The signs represent defects of blastogenesis (defective left-right patterning of the unpaired internal organs). Drosophila *cbp* was recently shown to function as a co-activator of transcription factor *ci (cubitus interruptus)* in *hedgehog* signalling: a molecular pathway establishing left-right asymmetry during blastogenesis. Thus the signs of Patients A and D may possibly represent a newly recognized pattern of RTS, or alternatively a novel contiguous gene syndrome.

Program Nr: 760 from the 1999 ASHG Annual Meeting

The recombinant 4 syndrome due to an unbalanced pericentric inversion of chromesome 4. *A. Battaglia*[1], *A.R. Brothman*[2], *J.C. Carey*[2]. 1) Inst Child Neurology & Psych, Stella Maris nst/Univ Pisa, Pisa, Italy; 2) Dept Peds/Div Med Genet, Univ of Utah Health Science Ctr, Salt Lake City, UT.

We have evaluated an informative patient with an MCA/MR syndrome consisting of global developmental delay, short stature, microcephaly, distinctive face, abnormal ears, iris coloboma and a congenital heart defect. His karotype shows 46,XY,rec(4) dup(4p) inv(4) (p14q35.1) mat, i.e. he has a partial 4p trisomy/distal 4q deletion due to an unbalanced pericentric inversion inherited from his mother. The 4p trisomy syndrome was originally described in 1970 [Wilson et al., Am J Hum Genet 22:679] in a very similar case with the same chromosome 4 inversion. Since then there have been over 75 cases published, mostly due to unbalanced translocations. Recent reviews suggest that the phenotype is hard to recognize clinically because the features are non-specific and the pattern is not as reproducible as in many chromosome syndromes. In contrast, 4p trisomy due to an unbalanced pericentric inversion of chromosome 4(p14q35); i.e. the recombinant 4 syndrome seen here, appears to be a discreet entity with relatively consistent features. This is similar to the rec 8 syndrome due to an unbalanced 8 pericentric inversion. Our patient is very similar to the original one of Wilson et al. In total there are 4 other kindreds described in the literature with this inversion, and the phenotype seems recognizable. Thus, we suggest that recombinant 4 syndrome is a discreet entity among 4p trisomy patients.

Program Nr: 761 from the 1999 ASHG Annual Meeting

Mitochondrial Diseases: A Recognizable Pattern of Systemic Disease. *C.A. Bay[1,2], M.A. Del Vecchio[1], G.L. Matika[1]*. 1) Div Medical Genetics, Children's Hosp of Pittsburgh, Pittsburgh, PA; 2) Dept. of Pediatrics, University of Pittsburgh, Pittsburgh, PA.

Symptoms of mitochondrial diseases are a consequence of abnormalities of the general function of mitochondria: to generate energy in the form of ATP, and to act as an effector of apoptosis. Despite the popular notion that mitochondrial diseases are always maternally inherited, they can be inherited as mendelian traits. The majority of mitochondrial genes are encoded in nuclear DNA, thus, they should be considered in diseases consistent with mendelian inheritance patterns. Previously recognized diseases with symptomatology typical of a mitochondrial disorder should be reevaluated to determine if they have a mitochondrial basis. By summing all symptoms present in individual family members, and considering the symptoms as if in one individual, we have been more effective in recognizing the typical systemic pattern of illness. Rarely does one individual demonstrate all the classical features present in the literature. A complete review of systems of family members is necessary to truly exclude this class of disorders. Symptoms to consider include: short stature, seizures, neuropathy, ataxia, apnea, progressive neurodegeneration, myoclonus, dementia, migraines, strokes, hemiparesis, cerebral palsy, dystonia, deafness, pseudoobstruction, cyclic vomiting, hepatopathy, increased liver function enzymes, chronic diarrhea, vague abdominal pain, irritable bowel syndrome, food intolerances, myopathy, diabetes, retinitis pigmentosa or degeneration, optic atrophy, lipomas and exercise intolerance. Frequent laboratory findings include lactic acidosis, abnormalities of electron transport chain, hyperalaninemia, ragged red fibers, mitochondrial proliferation, iron deposition, and hyper/hypothyroidism. However, the absence of these features does not preclude a mitochondrial disease. Examples of disorders which probably have a mitochondrial etiology are: OMIM # 165490: Optic Atrophy, Deafness, Ophthalmoplegia and Myopathy, and OMIM#234200: Hallervorden-Spatz Disease.

Program Nr: 762 from the 1999 ASHG Annual Meeting

The neurocognitive and psychosocial profile in children with a 22q11.2 deletion. *C.E. Bearden[1], M. Woodin[2], E. Moss[2], P. Wang[3], D. Driscoll[4], B.S. Emanuel[4], D.M. McDonald-McGinn[4], T.D. Cannon[1], E.H. Zackai[4].* 1) Dept of Psychology, University of Pennsylvania, Philadelphia, PA; 2) Dept of Child Psychology, Children's Seashore House, Philadelphia, PA; 3) Dept of Child Development, Children's Seashore House, Philadelphia, PA; 4) Div of Human Genet and Mol Bio, The Children's Hospital of Philadelphia, Philadelphia, PA.

Previous work has demonstrated that verbal skills are typically better preserved than non-verbal skills on both IQ and academic achievement testing in children with the 22q11.2 deletion (Moss et al., 1999). However, such measures are not sufficiently specific to determine a selective cognitive deficit in these children. In addition, the psychosocial profile of these children has not been well characterized. Here we present the first study to systematically evaluate these two issues in a cohort of children with 22q11.2 deletions. The psychosocial profiles of 58 children aged 5-17 with 22q11.2 deletions were assessed using parent ratings on the Child Behavior Checklist (CBCL). Internalizing problems and attention problems were significantly elevated in this cohort, regardless of IQ level or variance between verbal and performance IQ. A subset of these children (N=28) was evaluated with matched tasks of verbal memory (WRAML Verbal Learning) and visuospatial memory (CMS Dot Locations task). Regardless of IQ level or verbal-performance IQ split, all subjects displayed a selective deficit in visuospatial memory. These results indicate that children with a 22q11.2 deletion display a specific cognitive and psychosocial profile that is not adequately characterized by IQ tests. Findings will be instrumental in developing specific remediation strategies and are a first step towards localizing the CNS pathology in this syndrome.

Program Nr: 763 from the 1999 ASHG Annual Meeting

Third familial case report of the Ohdo syndrome in 2 Brothers. *B. Benzacken, B. Heron, J. Gaudelus, P. Bitoun.* *Embr-Cytogen & Biolog Reproduction, Neuro-Pediatrie, Pediatrie, Genet Medicale, CHU Paris-Nord, Hopital Verdier, BONDY, 93143cedex France.

Ohdo(1986) described 2 sisters and paternal cousin with mental retardation, heart defect, blepharophimosis & ptosis and hypoplastic teeth. 14 patients have been reported : Say & Barber 1987, Biesecker 1991, Maat-Kievit et al 1991, 1993, Clayton-Smith et al 1994, Melnick 1994, da Silva-Lopes&Guion-Dalmeida 1997, Rasmussen & Stromme 1998,Mhanni et al 1998; most of them sporadic.Some patients have deafness and all males have had cryptorchidism and/or scrotal hypoplasia.Dysmorphism includes broad flattened nasal tip & depressed bridge. Clayton-Smith et al found no evidence of microdeletion. Mhanni's patient and mother both have a balanced paracentric inversion of 9q, a common finding, suggestive of autosomal dominant inheritance with partial penetrance. We report 2 brothers, 5 and 3 years born to unrelated healthy parents presenting with mental retardation, blepharophimosis, blepharoptosis, ectopic testes in one and VSD in the other. Hypotonia and developmental delay were noted in the first year and only the older brother is starting with very few simple words. Both Patients have small ears and height near -1 s.d. They both have had an enlarged spleen resolved in the eldest and lymphocyte enzyme studies for storage disease which have been normal. The older boy has lighter hair on forehead concentrically darker from blond to dark brown towards vertex unrelated to sun exposure.They have had normal brain CT scan & MRI ,one has epilepsy with EEG anomalies responsive to valproate.Complete ophthalmologic examination is planned as well as hearing testing. Secondary amblyopia is a major risk of this blepharoptosis and should be detected and treated early. Both sibs have a 46XY high resolution karyotype without evidence of microdeletion. Parents are without any signs of blepharophimosis, ptosis, cardiac or genital anomalies but mother does have an unusually broad and prominent nasal tip. Both parents have normal high resolution karyotype. The mother consulted for genetic counseling with a new partner. The genetic counseling dilemma of recurrent Ohdo syndrome is approached.

Program Nr: 764 from the 1999 ASHG Annual Meeting

Isochromosome 20q mosaicism: extraembryonic or fetal in origin. *J. Bergoffen[1], D. Thibodeau[1], D. Edwards[2], L. Bros[1], B. Saxon[1], K. Nguyen[1], S. Owen[1], D. Sipos[1], X. Li[1].* 1) Genetics Dept, Kaiser Permanente Medical Grp, San Jose, CA; 2) Perinatology, KPMG, Hayward,CA.

Most reported cases of isochromosome 20q mosaicism (21/23) have resulted in normal pregnancy outcomes without confirming the i20q in fetal tissues. We present the second case of i20q mosaicism prenatally detected by amniocentesis but confirmed postnatally by interphase fluorescence in situ hybridization (FISH) in a baby with multiple congenital anomalies.

JH was a 3.4 kg (50%) product of a 38-week gestation delivered by NSVD to a 33 yo mother. Serial prenatal ultrasonography showed echogenic bowel, cardiac defects, and ventriculomegaly. Amniocentesis revealed 46,XX,i(20)(q10)[38 colonies]/46,XX[1]. Newborn exam was remarkable for length 5-10%, HC at 75%, micro/anophthalmia, and distal digit anomalies. Imagining studies revealed coarctation of the aorta with valve anomalies, anomalous ribs and vertebrae, and brain malformations. Support was withdrawn on day 3 of life and the infant died.

Standard cytogenetic analysis of 50 cells each from cultured blood and placental tissues failed to reveal i20q cells. However, interphase FISH using telomeric probes specific for 20pter and 20qter (Cytocell Limited) detected i20q in 15% of cells (30/200) in a direct preparation of the placental tissue but only 2% (4/202) in the cultured placental tissue and none in the blood culture (0/500). Interphase FISH analysis of a direct urine sample detected i20q in 27% of cells (54/200), confirming a true fetal mosaicism of i20q.

We suggest that true fetal mosaicism for i20q can have syndromic features such as ocular, skeletal, cardiac, and CNS anomalies. While most prenatally detected cases have been associated with normal outcome and suspected extraembryonic origin, the possibility of true fetal mosaicism for i20q must be carefully investigated. We caution that PUBS may not be helpful in prenatal cases. Our findings suggest that i20q cells may be selectively lost in culture. We propose that interphase FISH on directly prepared tissues is the method of choice for the confirmation of i20q.

Program Nr: 765 from the 1999 ASHG Annual Meeting

Noonan syndrome: a clinical and genetic study of 31 patients. *D. Bertola[1], C.A. Kim[1], S.M.M. Sugayama[1], L.M.J. Albano[1], J.D.A. Carneiro[1,2], E.A. D'Amico[2], C.H. Gonzalez[1].* 1) Dept. of Pediatrics, Instituto da Crianca, University of Sao Paulo, Sao Paulo, Brazil; 2) Dept. of Hematology, Hospital das Clinicas, University of Sao Paulo, Sao Paulo, Brazil.

Noonan syndrome is an autosomal dominant disorder comprising short stature, facial dysmorphisms, webbed neck, heart defect, cryptorchidism in the males, skeletal defects and abnormal hematological indices. In the present study, 31 affected patients, belonging to 26 families, were studied regarding their clinical and genetic characteristics. The isolated and familial propositus were separately analyzed from the affected relatives. Short stature was present in 69% of the propositus. The most common craniofacial dysmorphisms were ocular hypertelorism (62%), ptosis (58%) and downslanting of the palpebral fissures (42%), as well as high-arched palate and dental malocclusion. A webbed neck occurred in 42% of the propositus. The most frequent cardiac lesions, presented in 73% of the cases, were pulmonary valvar stenosis and hypertrophic cardiomyopathy. The eletrocardiogram frequently showed a superior QRS axis deviation. The abdominal ultrasound indicated the presence of hepatomegaly, splenomegaly or renal anomalies in less than 10% of the cases. Cryptorchidism occurred in 50% of the male propositus. The most common limb anomalies were prominent fetal pads on the fingers and toes and short and wide nails. The sternal deformity occurred in 54% of the propositus and the vertebral anomalies were observed in 23%. Abnormal hematological indices were present in 31% of our propositus, especially factor XI deficiency. The relatives showed less signs of the condition when compared to the propositus. In the 24 families in which the propositus' parents and sibs were evaluated, three (12.5%) had more than one affected, and so were considered familial cases. Therefore, the majority of the cases were sporadic.

Program Nr: 766 from the 1999 ASHG Annual Meeting

Congenital heart disease in Goldberg-Shprintzen syndrome. *M.G. Bialer, S.B. Ritz, M.E. Olson.* Dept Pediatrics, North Shore Univ. Hospital/NYU School of Medicine, Manhasset, NY.

A 5 wk old Guyanese male of mainly Asian Indian ancestry was seen because of Hirschsprung disease (HSCR) and congenital heart disease (CHD). He was the 3860g product of a 40 wk gestation born by primary C-section for transverse lie to a 35 yr old non-consanguineous mother who had normal amniocentesis. Baby was cyanotic at birth and was found to have a PFO and high muscular VSD resulting in congestive heart failure. At 11 days he developed vomiting, lethargy and abdominal distention and passed a bloody stool. Rectal biopsy revealed HSCR. He was hospitalized 3 times for pneumonia. His weight and OFC fell off the normal curve and follow-up visits identified a number of new problems including hypotonia, developmental delay and strabismus. Dysmorphology exams have revealed wide flat nasal bridge, epicanthal folds, broad bulbous nasal tip, pre-auricular pit, large ears, 5th finger clinodactyly, full lower lip, high arched palate, mild micrognathia with somewhat pointed chin, undescended testis, mild joint laxity, and somewhat hyperextensible skin. MRI scan of brain was normal. Sleep disturbance is a continuing problem and he will often stay awake all night. Blood chromosomes and FISH for Williams and Smith-Magenis syndrome deletions revealed no abnormalities. Findings are suggestive of Goldberg-Shprintzen syndrome (GSS). GSS is a rare autosomal recessive condition initially described in siblings with HSCR, submucous cleft palate, microcephaly, short stature, dysmorphic features and developmental delay. At least 23 cases with similar features have subsequently been described. Syndrome nosology may evolve. Although earlier cases of GSS had submucous cleft palate and iris coloboma, few subsequent cases have. CHD was not described in the earlier cases, but this is the 8th subsequent case with CHD. Other cases have had ASD, ASD/PDA, VSD, PS, PA/VSD, ASD/TOF, and PDA/PPS. Only 3 cases have lacked HSCR, and 2 were the sibs of an affected child with HSCR. Since all the other features, except developmental delay, have been variable, it seems probable that HSCR may also be, and the condition may be more common than previously thought.

Program Nr: 767 from the 1999 ASHG Annual Meeting

Variability in the behavior phenotype of the de Lange syndrome. *G.M. Bibat, G.V. Raymond.* Div Neurology & Dev Medicine, Kennedy Krieger Inst, Baltimore, MD.

The de Lange syndrome (dLS) is a developmental malformation syndrome characterized by mental retardation, growth delay, distinctive facial features, and limb reduction defects. A behavior profile described includes poor social relatedness, aggressive, disruptive, and self- injurious behavior. While a well-recognized condition, there may be considerable variability in its phenotypic expression and the full range of behavioral abnormalities has not been delineated. A retrospective review from 1994-1999 was conducted. Six patients with dLS were identified. They all had the characteristic facial features which included microbrachycephaly, synophrys, anteverted nares, triangular nasal tip, crescent-shaped mouth, and micrognathia. In addition, all had postnatal growth deficiency (4 of the 6 patients had prenatal growth retardation) and limb anomalies ranging from brachydactyly, camptodactyly, syndactyly, clinodactyly, to phocomelia of the upper extremity. Four of the six had karyotypes which did not reveal any abnormalities. All six had cognitive and behavioral abnormalities. Mental retardation ranged from mild to profound. One patient had mild articulation disorder while another was nonverbal (used sign language). Three of the 6 had some degree of hearing loss. Behavioral diagnosis included ADHD, oppositional defiant disorder, obsessive compulsive disorder, aggressive and disruptive behavior, stereotypies, and self-injurious behavior. Treatment included in most cases stimulant medications for inattention, mood stabilizers, and neuroleptic agents for aggression when appropriate. Response to medication was variable. Two of the 6 required admission to a psychiatric behavioral unit for aggressive and self-injurious behavior. Their behavior was described as explosive physical outbursts; destructive head banging episodes; and life threatening elopement. Both had severe to profound mental retardation. One patient had severe pica involving inedible objects (screws, lids, and coins) requiring surgical removal. The behavioral profile of dLS appears to correlate with the severity of their clinical features and cognitive potential, but additional study is necessary to determine the full range of behavior and most effective therapeutic approach.

Program Nr: 768 from the 1999 ASHG Annual Meeting

Diphallia: Case Report and Discussion of Etiologic Mechanisms. *R.D. Blackston.* Div Med Gen, Dept Pediatrics, Emory Univ Sch Medicine, Atlanta, GA.

Penile duplication (PD) is a very rare congenital anomaly with an estimated occurrence of 1 per 5 million live births in the United States. PD is rarely seen in isolation but as part of a cluster of congenital anomalies: spina bifida, vertebral, renal, anorectal and hindgut duplication. It is thought that these clusters of anomalies may be the result of an insult to caudal cell mass at approximately day 23 to 25 of gestation. We report a 6 week old Mexican-American male with diphallia seen in one of our genetic outreach clinics. He was an alert, handsome infant who had two complete penises with two crura on each penis. The anus was proximal and to left of the midline. There was a mass on the right buttocks consistent with meningocoele, confirmed by MRI scan. Plain films showed abnormalities of the lumbar spine with some tethering of the cord. There were two kidneys each with a urethra draining into separate bladders. The mechanism of PD and the associated anomalies in our patient may be explained by duplication of the caudal body elements of mesodermal origin. Between 21 and 35 days of gestation mesoderm migrates caudally separating the urogenital sinus from the rectum and the genital tubercle, later the phallus results from merging of the paired columns of mesoderm. The diphallia could also be the result of defect in migration of mesoderm through the cloacal membrane early in embryogenesis. Some initial work on Drosophila suggests a defect in homeobox genes as the mechanism of diphallia. We report another case of the rare anomaly diphallia. Significant emotional trauma can be spared frightened, confused parents by careful and continued support that their sons will have full productive lives.

Program Nr: 769 from the 1999 ASHG Annual Meeting

Microcephaly-Micromelia Syndrome in the Cree Indians of Northern Saskatchewan. *P.M. Blakley[1, 2], L.B. Holmes[1], R.E. Casey[2]*. 1) Genetics & Teratology Unit, Massachusetts General Hosp, Boston, MA; 2) Dept Pediatrics, Royal University Hosp, Saskatoon, Saskatchewan, Canada.

Microcephaly-Micromelia Syndrome (MMS) is a constellation of lethal, congenital malformations observed in the offspring of the Cree First Nation people living in LaRonge, Saskatchewan. It was first recognized in the 1950's and at that time was responsible for 20% of neonatal deaths among the Cree people. It has not been observed in the Caucasian population of LaRonge or in the offspring on other First Nation reserves. MMS is inherited in an autosomal recessive manner.

Twenty-two infants with MMS were identified through medical records at Royal University Hospital, Saskatoon. All infants were noted to be severely growth retarded with birth weights, lengths and head circumferences <3rd%. Microcephaly and cerebral dysgenesis were observed in all infants. Characteristic facial features, including prominent nose, micrognathia, microstomia, microphthalmia and craniosynostosis, were reported in 63% of infants. All infants had evidence of limb reduction deformity, manifested as aplasia/hypoplasia of the radius/ulna and absent digits. 29% of affected infants had three or less digits per hand. The arms were more severely affected than the legs. Other defects included congenital heart (58% of infants), renal (42%), musculoskeletal (33%), endocrine (33%), gastrointestinal(25%), respiratory (25%) and genital defects (17%).

The differential diagnosis of MMS includes Microcephaly Osteodysplastic Primordial Dwarfism I and III, Seckel Syndrome and Cerebro-Oculo-Facial-Skeletal Syndrome. While the facial features are similar to MMS, limb reduction deformities and lethality are not characteristic of these syndromes.

Molecular DNA studies, utilizing homozygosity testing of markers associated with microcephaly and limb defects, are currently in progress to define the chromosomal locus associated with MMS.

The present study describes a unique syndrome observed in the Cree people. Delineation of the molecular basis for MMS will allow for carrier testing and prenatal diagnosis.

Program Nr: 770 from the 1999 ASHG Annual Meeting

RNASEH1 gene in 17p11.2 deleted in Smith-Magenis syndrome (SMS). *J.K. Blancato[1], T. Lin[1], G. Veytsman[1], R.J. Crouch[2], S.M. Cerritelli[2], A. Gropman[3], A.C.M. Smith[1,3].* 1) Institute Molec. & Human Genetics, Georgetown Univ Medical Ctr, Washington, DC; 2) Laboratory of Molec. Genetics, NICHD/NIH, Bethesda, MD; 3) Med. Genetics Branch, NHGRI/NIH, Bethesda, MD.

Smith-Magenis syndrome (SMS) is a contiguous gene deletion syndrome of 17 p11.2 associated with a complex phenotype of characteristic physical features, clinical signs of peripheral neuropathy, developmental delay, and neurobehavioral problems, including sleep disturbance and self-injurious behaviors. Estimated to occur in 1/25,000 births, more than 150 cases have been identified worldwide from a diversity of ethnic groups. While most patients have a common SMS deletion interval spanning 4-5Mb, deletions have ranged from <2 to >9. Recently, the RNASEH1 gene was mapped to 17p11.2 region (Cerritelli & Crouch, 1998). Subsequent sequencing data, including lack of intron sequences, suggest this is a putative pseudogene. Although the RNASEH1 gene is in the region, it does not appear to be within the critical SMS interval. It is deleted in 2 of 10 SMS patients studied by FISH with a plasmid probe for the RNASEH1 gene. Clinically, these two females appear to have more severe involvement of the peripheral nervous system than classical SMS. Molecular studies confirm they have larger deletions extending distally, including deletion of the PMP22 gene and D17S122 and D17S261. Case 1, a female previously described as Patient #118-484 by Zori et al.(1993), now demonstrates progressive nerve conduction abnormalities consistent with bilateral peroneal (ankle) and median nerve (wrist) compression neuropathy. While Case 1 represents the only known case of an inherited deletion from a mosaic carrier mother, Case 2 has a de novo deletion of paternal origin based on DNA marker analysis. This is the first report of a possible pseudogene in the SMS region in the growing literature describing low copy repeat sequences and their association with chromosomal rearrangements, and specifically, microdeletion syndromes. Fiber FISH with known SMS markers is currently being used to better order the RNASEH1 gene and further define the breakpoints in these two children. *Cerritelli SM and RJ Crouch (1998) Genomics 53(3):300-307.*.

Program Nr: 771 from the 1999 ASHG Annual Meeting

Male to male transmission of Costello syndrome consistent with autosomal dominant inheritance. *N.M. Bodkin[1], E.S. Mortimer[2], L.A. Demmer[1]*. 1) Department of Pediatrics, Univ of Massachusetts Memorial Health Care, Worcester, MA; 2) Department of Orthopedics, Univ of Massachusetts Memorial Health Care, Worcester, MA.

Costello syndrome is a rare disorder involving mental retardation and multiple congenital anomalies. At least 52 patients have been reported with almost all cases presenting as isolated incidents in a family. Therefore the inheritance pattern has not yet been identified. Here we present the case of a 12 year old male with classic Costello syndrome whose father has many of the features of this disorder in an asymmetric pattern on his body suggestive of mosaicism.

Consistent with the marasmic phase of Costello syndrome, our patient's father has a history of feeding problems and failure to thrive in early childhood. Hyperkeratosis, seen in 88% of reported cases of Costello syndrome, was noted on both of his hands and was extreme on his left foot but absent on his right. Also remarkable was the asymmetrical presentation of anal papillomata which were present on the right but not left perianal region. Nasal papillomata had previously been removed. His affable personality, history of low normal intelligence scores, hyperpigmentation, thick ear lobes, increased AP diameter in the chest and patches of curly in his otherwise straight hair are all features consistent with Costello syndrome.

This apparent father to son transmission suggests that Costello syndrome is inherited as an autosomal dominant disorder. We predict that the father of our patient is a mosaic for Costello syndrome, probably the result of a post-zygotic mutation. Many of the previously reported affected males have a history of undescended testes and several of the females have a history of delayed puberty or amenorrhea. This suggests that infertility may be a feature of Costello syndrome and may account for the fact that there have been no other reported cases of vertical transmission of the disorder. Careful examination of the parents of all affected individuals is recommended when considering recurrence risk for Costello syndrome.

Program Nr: 772 from the 1999 ASHG Annual Meeting

Shprintzen-Goldberg syndrome (SGS): Report of 2 affected half siblings. *A. Bogdanow[1], P. Levy[1], A. Shanske[1], R.J. Shprintzen[2], R. Marion[1]*. 1) Ctr for Congenital Disorders, Montefiore Medical Ctr, Bronx, NY; 2) Communications Disorders Unit, Health Science Center at Syracuse, Syracuse, NY.

First described by our group in 1982, SGS combines craniosynostosis, MR, characteristic facial features, and other skeletal and connective tissue defects. Although AD inheritance is supported by a mutation in FBN1 in at least one of the clinical subtypes of this disorder, SGS has occurred in 3 sibs born to unaffected, non-consanguineous parents (Ades et al., AJMG , 1995). This has raised the possibility of either AR inheritance or AD with germinal mosaicism in one of the parents. We describe affected half sibs, an observation supporting the latter hypothesis.

Patient 1, the 3.86 kg product of an uncomplicated pregnancy born to a 20 yo primigravida, was admitted to the ICU at 6 weeks because of obstructive apnea (requiring tracheostomy) and FTT (necessitating a gastrostomy).. The infant had brachyturricephaly, ocular proptosis with downward obliquity and hypertelorism, low-set posteriorly rotated ears, high arched palate, pectus excavatum, and arachnodactyly of fingers and toes. A 3D CT scan revealed partial closure of the sagittal, lambdoidal and coronal sutures and ventriculomegaly. A diagnosis of SGS was made. Patient 2, his half sib, was the 3.64 kg product of his mother's second pregnancy. He had many of the same craniofacial and digital anomalies. In addition, he had malrotation of the intestines and an aberrant right subclavian artery. Examination of the mother and both fathers revealed no features of SGS or of a connective tissue dysplasia.

The occurrence of SGS in 2 half sibs born to unaffected, non-consanguineous parents provides further support for the conclusion that SGS has AD inheritance with germinal mosaicism. Five of the 21 patients described to date are the result of germinal mosaicism indicating the frequency of this occurrence in SGS. This must be kept in mind when these families are counselled.

Program Nr: 773 from the 1999 ASHG Annual Meeting

PAX3 missense mutations (G99S and R270C) in the original patient described with Klein-Waardenburg (WS3) syndrome. *A. Bottani, S.E. Antonarakis, J.L. Blouin.* Division of Medical Genetics, Geneva University Hospital, Geneva, Switzerland.

In 1947, David Klein in Geneva described (Arch Jul Klaus Stiftg 1947; 22: 336) a 10 year-old female with a previously unreported condition consisting of "partial albinism, deaf-mutism, osteomyodysplasia, multiple congenital joint contractures, and other congenital malformations". Parents were non consanguineous and phenotypically normal. This condition has since been called Klein-Waardenburg or Waardenburg type 3 (WS3) syndrome.We reexamined the 62 year-old proposita at her home, where she lives independently despite her striking physical handicaps. Sequence analysis of PCR-amplified genomic DNA from this patient revealed two PAX3 gene missense mutations which occured in conserved aminoacids of important PAX3 domains: G99S in exon 2 (encoding the paired box) and R270C in exon 6 (encoding the homeobox). Mutations of the same residues have previously been described in classical Waardenburg syndrome type 1 (WS1) heterozygotes. The origin (de novo vs inherited?) of the patient's mutations cannot be determined, as parents are deceased. This study confirms that WS3 is due to PAX3 mutation(s) and is thus allelic to WS1. It also shows that compound heterozygosity and, as previously reported, homozygosity for PAX3 mutations can lead to a severe, but compatible with long term survival, Waardenburg syndrome phenotype.

Program Nr: 774 from the 1999 ASHG Annual Meeting

Split cognitive function in an intelligent adult with Joubert syndrome despite dramatically hypometabolic cerebellum. *D.G. Brooks[1,2], M.M. Kurtz[3], J. Farmer[1], G. Liu[4], L. Harper Mozley[3], R.C. Gur[3], C.A. Friedrich[1]*. 1) Div. Medical Genetics; 2) Dept Genetics; 3) Neuropsychiatry Program; 4) Depts of Neurology and Ophthalmology, Hosp Univ Pennsylvania, Philadelphia, PA 19104.

Hypoplasia of the cerebellar vermis and adjacent brainstem/midbrain are characteristic of Joubert syndrome (JS). These anatomic deficiencies underlie the clinical features of JS: hypopnea, hypotonia, ataxia, developmental delay and abnormal eye movements. In a rare, high functioning adult with JS we investigated cerebellar metabolic activity as well as visual and cognitive function. The proband is a 25 year old female with coarse features, macrocephaly, synophrys and mandibular prognathism. She had generalized hypotonia, dysmetria, ataxic/shuffling gait and slow, dysarthric speech. By history there was apnea, hypotonia, delayed motor development, ataxia, tongue thrusting, dysconjugate gaze and preaxial polydactyly. She completed 1 year of college. MRI revealed hypoplastic superior cerebellar vermis, an enlarged 4th ventricle and mild cortical volume loss. PET scan revealed marked, uniform cerebellar hypometabolism. Fundoscopic examination was normal and an ERG was attenuated with compromised rod and slightly impaired cone responses. Corrected visual acuity was 20/400 OD and 20/80 OS; visual fields were constricted peripherally OU. There was ptosis, diffuse ophthalmoparesis and supranuclear gaze palsy. In contrast to severe MR in JS children, selective cognitive difficulties were apparent on neuropsychological testing. Strengths included general knowledge, expressive vocabulary, verbal comprehension/retention. Weaknesses included problem solving, visual memory and sensory-motor testing. Overall borderline intellectual function was evident by the following IQs: full scale 77, verbal 88 and performance 68. Although the cerebellar deficit seen on MRI is restricted to vermian hypoplasia this first ever PET scan of a JS patient shows hypometabolism of the entire cerebellum. The resulting severe motor dysfunction as well as visual/perceptual difficulties can mask considerable intelligence in JS. This case has implications for prognosis, genetic counseling and education of JS patients and families.

Program Nr: 775 from the 1999 ASHG Annual Meeting

Mild clinical features of 18q deletion and trisomy 19q seen in a child with a novel 18;19 translocation. *T.C. Brown[1], C. Kozma[2], J. Meck[3].* 1) Genetic Studies Section/LSB, NIAMS/NIH, Bethesda, MD; 2) Department of Pediatrics, Georgetown University Medical Center, Washington, D.C; 3) Department of OB-GYN, Georgetown University Medical Center, Washington, D.C.

Monosomy 18q first described in 1964, is well defined clinically. Patients with 18q- syndrome have been reported with the following: growth and mental retardation, facial dysmorphism, and multiple congential anomalies (MCA). The phenotypes are highly variable with a general correlation between the size of the deletion and the phenotypic severity. Cytogenetically the deletions appeared to include 18q21.2-18qter. Molecular analysis showed that some deletions were complex interstitial rearrangements. The critical region has been reported to be 18q23. Fewer chromosome 19q duplications have been reported in the literature. Along with cardiac anomalies, the phenotypic anomalies are similar to 18q- syndrome. 19q13 terminal duplications have been reported. No genes identified in these two regions are the primary cause of the phenotypic abnormalities. We describe a unique case involving a 4 year old girl with both partial monosomy 18q and partial trisomy 19q [46,XX,der(18)t(18;19)(q21.3;q13.42)mat]. We are not aware of similar cases in the literature. She presents with growth retardation, repaired ASD, repaired abdominal wall defect, repaired anteriorly placed anus, partial hearing loss, and delayed myelination documented on MRI. She has mild midface hypoplasia, upslanted palpebral fissures, bilateral nystagmus, and borderline low set ears. In addition, she has bilateral clinodactyly, small hands and feet, altered palmar creases, and hypotonia. She is currently receiving growth hormone injections for growth hormone deficiency and delayed bone age. She is independent in feeding and ambulation and communicates verbally by using one-two word sentences. These mild phenotypic features are surprising since the 18q monosomy is rather large and is coupled with 19q trisomy. This novel case should provide more information for recurrence risks and allow for refinement of the phenotypic map of these chromosomal regions.

Program Nr: 776 from the 1999 ASHG Annual Meeting

Human HOX-A1 gene and hindbrain malformations. *V. Capra[1], A. Moroni[1], P. De Marco[1], A. Faiella[2], E. Boncinelli[2], A. Cama[1]*. 1) Lab Servizio Di Neurochirurgia, Inst Scientifico G Gaslini, Genova, Italy; 2) DIBIT,Ospedale San Raffaele, Milano,Italy.

During hindbrain development, Hox genes provide regional specification and morphogenetic identity to the rhombomeric segments. Functional inactivation of Hox-a1 gene results in complete or near complete deletion of rhombomere 5 (r5) and severe reduction of r4 suggesting that Hoxa1 is acting in maintenance and/or generation of hindbrain segments. Hox-b1 instead is restrictly expressed in rhombomere 4 and its role is to give segmental identity to this region, that will give rise to the rhomboencephalon. The Hox-b1 and Hox-a1 double mutant phenotype seems to confirm functional interaction of these genes in determining hindbrain structure, cranial nerves and second pharingeal arch derivatives patterning. Furthermore Hox-a1-/- homozygotes have malformations of the basioccipital and exoccipital bones of the skull and of the external, middle and inner ear. Our patients present variable complex phenotypes including deafness, cranio-facial and vertebral anomalies (like Chiari I and Dandy-Walker malformation) that closely resemble Hox-a1 and/or Hox-b1 mutants mice. We assessed the presence of genomic mutations HOXA1 genes by means of the Single Strand Conformation Polymorphism (SSCP) technique in 30 sporadic cases of hindbrain malformations. We failed to find any mutated fragment on the coding region of the gene. Different paralogous homeobox genes expressed in the hindbrain will be analyzed. Acknowledgement: This project has been supported by Comitato Promotore Telethon E. 795.

Program Nr: 777 from the 1999 ASHG Annual Meeting

A NOVEL N114D TWIST MUTATION IN A CROUZON-LIKE PATIENT. *C. Carbonara[1], L. Sbaiz[2], L. Genitori[3], P. Peretta[3], F. Mussa[3], C. Nurisso[3], G. Restagno[2], S. Belli[4], G.B. Ferrero[1]*. 1) Dept Pediatrics, Univ di Torino, Torino, Italy; 2) Medical Genetic Lab., OIRM, Torino; 3) Pediatric Neurosurgery, OIRM, Torino; 4) Genetic Counselling Service, Trento.

Craniosynostosis, the premature closure of one or more cranial suture occur in about 1/2500 infants and represent an heterogeneous group of disorders. Recently mutations in the TWIST gene, a helix-loop-helix transcription factor, have been reported in patients presenting with Saethre-Chotzen syndrome, characterized by facial asymmetry, ptosis, hypertelorism, brachydactyly and cutaneous syndactylies. Here we report a novel mutation of the TWIST gene in a patient presenting with brachycephaly, severe ocular proptosis and no limb anomalies, features not typical of Saethre-Chotzen syndrome, rather of Crouzon syndromes phenotype. The A to G transition at position 340 leads to the aminoacid change N114D in the DNA binding domain and presumably alters the affinity of the mutated TWIST protein for its DNA target sequence. The presence of the mutation has been confirmed both by direct sequencing and by digestion with the MscI restriction enzyme. The phenotypically normal parents have been investigated both by sequencing and by MscI digestion and found normal. The patient has been also screened for mutation in the FGFR2 gene (exons IIIa and IIIc) and FGFR3 (exon 7) and found normal. This newly described mutation is likely to be pathological for the following reasons. 1) it represents a de novo mutation; 2) missense mutation of nearby codons have been already reported in Saehtre-Chotzen patients; 3) asparagine at position 114 is highly conserved in other species. The description of this patient shows that heterogenous conditions might be associated with TWIST mutations, as it has been already observed with FGFR2 and FGFR3 mutations.

Program Nr: 778 from the 1999 ASHG Annual Meeting

Molecular analysis of a hermaphrodite patient with triploid-diploid mosaicism. *A.K.J. Chan[1], T.L. Stockley[2], I. Teshima[2], P.N. Ray[2], D. Chitayat[3]*. 1) Clinical Genetics, The Hospital for Sick Children, Toronto, ON; 2) Pediatric Laboratory Medicine, The Hospital for Sick Children, Toronto, ON; 3) The Prenatal Diagnosis Program, University of Toronto, Toronto, ON, Canada.

Triploid-diploid mosacisim is an uncommon condition and its association with hermphroditism has rarely been described. We present a triploid-diploid mosaic patient with hermaphroditism who was investigated using molecular microsatellite analysis. The patient presented at birth with ambiguous genitalia and was found to have a left-sided ovotestis and a right-sided ovary, each associated with a fallopian tube. A normal uterus and vagina were present. Cytogenetic analysis indicated a normal 46XX female karyotype in blood (n=50), and interphase nuclei (n=200) were negative by FISH for the Yp11.2 probe (Oncor). A 46XX/69XXY mosaic karyotype was found in the ovotestis and skin fibroblasts. Cytogenetic analysis estimated the percentage of triploid cells to be 36% in ovotestis and 33% in skin.

Molecular STR analysis was used to determine the formation of the diploid-triploid mosaicism. DNA was extracted from the patient's blood and ovary tissues and from the triploid-diploid mosaic ovotestis and skin tissues, as well as from parental blood samples. Analysis of microsatellite polymorphisms from nine different chromosomes indicated that the triploid-diploid mosaic tissues contained two paternal markers at certain loci, suggesting that the triploid cells were diandric in origin. The triploid cells likely originated from dispermy as indicated by random assortment of paternal microsatellite markers to the triploid-diploid mosaic tissues. Possible mechanisms for triploid-diploid mixoploidy include fusion of a diploid and a triploid zygote to produce a chimera, or alternatively mosaicism resulting from loss or gain of a haploid genome. Molecular results suggest that the most likely mechanism for triploid-diploid mixoploidy in this patient is not chimerism but is mosacisim resulting from gain or loss of a paternal genome.

Program Nr: 779 from the 1999 ASHG Annual Meeting

Mapping of the Translocation Breakpoint in a Patient Displaying Morbid Obesity and Features of the Coffin-Siris Syndrome. *F.F. Chehab[1], M. Golabi[1], J. Qiu[1], G. Magrane[1], P. Cotter[2].* 1) Departments of Laboratory Medicine and Pediatrics, University of California, San Francisco, CA; 2) Division of Medical Genetics, Children's Hospital, Oakland, CA.

The search for genes that underlie predisposition to obesity will improve understanding of body weight regulation and increase our ability to treat obesity-associated disorders. Coffin-Siris syndrome (CSS) is a multiple malformation syndrome with growth deficiency, undetermined mode of inheritance and an unknown molecular basis. We report an 11 year-old female who presented initially with features resembling those seen in Coffin-Siris syndrome. Hypertrichosis, sparse scalp hair, coarse facial features, hypoplastic fifth fingernails and mental retardation were observed in early infancy. Karyotype revealed 46, XX and a de novo translocation between 7q32 and 22q12. At 11 years, she developed severe obesity, which has never been reported to be associated with CSS. An individual diagnosed with CSS was previously found to carry a translocation at 1q21 and 7q34. Therefore, it is likely that the region around 7q32-34 might underlie the gene for CSS. Interestingly, the same chromosomal region includes the leptin gene, which is mutated in few morbidly obese individuals. Although it is possible that her obesity may be distinct and separate from her multiple malformation syndrome and be due to disrupted genes on both derivative chromosomes, it is also conceivable that both features could represent disruption of a gene on chromosome 7. Structural rearrangement of the leptin gene was ruled out based on a normal genomic Southern blot probed with leptin cDNA and a leptin level of 32 ng/ml, which correlated, with her BMI of 34.4. Yeast artificial chromosomes (YACs) which bracket the translocation breakpoint were assembled from chromosome 7 and spanned approximately 12 cM. Fluorescent in situ hybridization (FISH) with YACs from the contig and chromosome spreads from this patient allowed us to narrow down the critical region to 500 kb. Current efforts aimed at molecular cloning of the breakpoint region will yield genomic fragments from chromosomes 7 and 22 and reveal insights into the nature of interrupted genes.

Program Nr: 780 from the 1999 ASHG Annual Meeting

Neurological and dysmorphic features in a family with brachial plexus neuropathy. *A. Chen, M. Kovach, V. Kimonis.* Div. of Genetics & Metabolism, Dept Pediatrics, Southern Illinois Univ, Springfield, IL.

Hereditary neuralgic amyotrophy (MIM 162100) or familial plexus neuropathy is an autosomal dominant disorder characterized by the onset of recurring episodes of back pain and muscle weakness early in childhood. Affected individuals also have hypotelorism or closely spaced eyes. Although the molecular basis for this disorder remains unknown, genetic linkage has been established to chromosome 17q24-q25 (Pellegrino et al. 1997; Stogbauer et al. 1997). We report a four-generation family presenting with autosomal dominant brachial plexus neuropathy. The proband a 16 y. old male presented with pain and atrophy of his left deltoid, suprascapular, rhomboid muscles (causing winging of his scapular) and abductor pollicis brevis muscle. A further episode of pain in his right arm did not lead to permanent loss of function. Dysmorphic evaluation in him revealed hypotelorism and small palpebral fissures. His father age 47 y. was asymptomatic, however had hypotelorism. His paternal uncle age 49 y. has had a history of recurrent episodes of pain preceding weakness in his arms since the age of 17 y. He has marked wasting of the muscles in his hands and similar facies. The paternal grandmother developed recurrent paralysis in both arms since a pregnancy at the age of 24 y. She also developed weakness of her legs, became bedbound this leading to her death at the age of 54 y. Genetic linkage analysis with chromosome 17q24-q25 markers was performed in six family members (2 affected, 1 carrier, 2 unaffected, and 1 spouse). The power of the pedigree was not sufficient to generate significant LOD scores (maximum LOD score 0.109 at a recombination fraction = 0.00 for marker D17S802), however haplotype analysis with markers D17S802, D17S939 and D17S1603 revealed a shared haplotype among affected individuals. This study further confirms the lack of heterogeneity of this interesting familial disorder causing facial dysmorphisms and recurrent episodes of neuropathy.

Program Nr: 781 from the 1999 ASHG Annual Meeting

Fragile X Premutation Status in a Male with Mental Retardation. *E.J. Chernoff[1], M. Nunes[2], B. White[3], J. McClellan[2], E. Coll[4], K. Maggio[5], S. Levin[4].* 1) Heritable Disorders Branch, NICHD, National Institutes of Health, Bethesda, MD; 2) USAF Medical Genetics Center, 81st Medical Group, Keesler AFB,MS; 3) Quest Diagnostics, Nichols Institute, San Juan Capistrano, CA; 4) Department of Pediatrics &; 5) Dept of Dermatology, Walter Reed Army Medical Ctr, Wash. D.C.

Fragile X syndrome(FXS) is the most common form of inherited mental retardation(MR). It is caused by expansion of CGG repeats in the 5' exon of the FMR1 gene, known as full mutation(FM), and subsequent methylation of the CpG island. Mosaicism of gene repeat length or methylation has been documented and tissue heterogeneity has been demonstrated(AJMG 84:233,1999).

We report a 8 yr old male with moderate MR, the product of a term pregnancy complicated by oligohydramnios, growth retardation and placental calcifications. History is notable for bilateral cryptorchidism, recurrent otitis media, gastroesophageal reflux and strabismus. His nonconsanguineous parents had two prior first trimester pregnancy losses; there was no family history of MR. Exam noted rocker bottom feet with valgus deformity, right simian crease and hyperpigmentation on the abdomen and thighs. Our blood karyotype was found to be 46,XY (550 band level). Our analysis of fibroblasts from biopsies of hyperpigmented and normally pigmented skin was also 46,XY. However, fragile X analysis from blood and the 2 fibroblast sites showed a premutation(PM) of 100-165 repeats compared to a maternal PM in blood of 70-90 repeats by Southern blot. Methylation was normal .

Although our patient had MR and other features consistent with FXS and not due to a cytogenetic abnormality, a cell population with FM could not be demonstrated in blood or skin. While MR in this case may be caused by factors other than fragile X status, the finding of a PM expansion beyond that of his mother suggests the two findings may be related. Expansion to a FM could have occurred in the brain or other tissues not studied. Even though a FM was not detected in our patient, similar studies of skin fibroblasts should be considered to rule out this possibility in dysmorphic males with unexplained MR who have a PM and normal karyotype in peripheral blood.

Program Nr: 782 from the 1999 ASHG Annual Meeting

Rubinstein-Taybi syndrome with hepatoblastoma. *J. Claus, B. Kousseff, J. Ranells.* USF Regional Genetics Program, Tampa, FL.

We report a two-year old male patient, with Rubinstein-Taybi syndrome, phenylketonuria and hepatoblastoma. The patient, GP, was the first child of a 35-year old gravida 2 para 0-0-1-0 mother. The mother has mitral valve prolapse and left conductive hearing loss secondary to abnormal ossicles. Prenatal triple marker screen indicated an increased risk for Down syndrome. Amniocentesis was declined. Fetal sonograms suspected a Dandy-Walker variant with no hydrocephalus and severe IUGR. Delivery was at thirty-two weeks gestation by emergency Cesarean section for pre-term labor and vaginal bleeding secondary to subchorionic hematoma. Apgar scores were 6/8; birth weight was 1282 g (75th centile; corrected for gestation), birth length was 38 cm (5th centile; corrected), and OFC was 27 centimeters (5th centile; corrected). The umbilical cord had three vessels and was thick. A large PDA was diagnosed. He required a ventilator and phototherapy. EKG was abnormal with right axis deviation. Genetics consult revealed a prominent nose, pencil-like eyebrows, low-set ears, hypoplastic nipples, undescended testes, broad first digits and angulated thumbs. Hypotonia was noted. The impression was Rubinstein-Taybi syndrome. Indomethacin reduced the PDA. Newborn metabolic screening at 6 days of age showed a phenylalanine level of 8 mg/dl. Follow up level was 62.2 mg/dl at 15 days of age. He was diagnosed with classic PKU. Neopterin, biopterin and dihydropteridine reductase levels were normal. Karyotype was 46,XY. Ophthalmalogic evaluation was normal. Cranial sonograms showed no posterior fossa abnormality. MRI scan showed increased signal on the periventricular and subcortical white matter. The finding was considered nonspecific and possibly consistent with prematurity. Renal sonogram showed slightly small kidneys with mild left hydronephrosis. At two years of age GP presented with chronic constipation and feeding difficulties as well as low grade fevers and an enlarged liver. A surgical biopsy showed hepatoblastoma. Although a variety of rare tumors have been reported in individuals with Rubinstein-Taybi syndrome, this is the first report of hepatoblastoma in this condition.

Program Nr: 783 from the 1999 ASHG Annual Meeting

A novel WT1 mutation in Frasier syndrome: Support for the concept of the "WT1 spectrum disorder". *C.L. Clericuzio[1], F. Byrn[1], V. Huff[2]*. 1) Univ New Mexico Sch Medicine, Albuquerque, NM; 2) UT MD Anderson Cancer Center, Houston, TX.

The WT1 gene acts as both a regulator of normal genitourinary development and a tumor suppressor gene. Germline mutations of WT1 have been found in individuals with a wide range of genitourinary malformations and Wilms tumor (WT) risk. The clinical triad of male genital ambiguity, early onset nephropathy and high WT risk has been identified as the Denys-Drash syndrome (DDS). Greater than 95% of DDS patients have germline WT1 mutations, which are often missense mutations in the zinc finger coding regions. A somewhat distinct pattern of genitourinary malformation, characterized by complete sex reversal in XY individuals, later onset nephropathy and low WT risk has been termed the Frasier syndrome (FS). Approximately 20 FS individuals have been reported to have WT1 donor splice site mutations in intron 9, predicted to produce an altered ratio of alternative splice products.

We report a FS patient with a novel WT1 point mutation at the 5' splice junction of exon 10, which is also predicted to alter WT1 splicing. Our patient's genitourinary phenotype is atypical for FS in that she had clitorimegaly and a rudimentary testis.

This patient illustrates blurring of the distinction between DDS and FS genitourinary phenotypes. Likewise, while DDS and FS have been historically distinguished from one another by high WT risk in the former, and nil risk in the latter, Barbosa (1999) report a FS patient with WT and a typical mutation. Further compounding the question of WT risk in individuals with genitourinary anomalies, is the identification of protein truncating WT1 mutations in boys with WT and cryptorchidism/hypospadias (CH), who have no nephropathy.

In light of the lack of clear distinction between DDS/FS/CH phenotypes associated with WT1 mutations, we suggest that the term "WT1 spectrum disorder" be adopted to encompass the range of disorders which can arise from alterations in the WT1 gene. Within the spectrum, there remain general phenotype-genotype correlations.

Program Nr: 784 from the 1999 ASHG Annual Meeting

PROP1 gene analysis in familial and sporadic CPHD. *J.D. Cogan[1], J.A. Phillips[1], J.L. Carneal[1], L.K. Hedges[1], D.A. Doyle[2]*. 1) Dept Pediatrics/Genetics, Vanderbilt Univ, Nashville, TN; 2) Temple University, School of Medicine, Philadelphia PA.

Combined pituitary hormone deficiency (CPHD) has an incidence of ~1/8,000 births with ~10% of cases having an affected first degree relative. Affected individuals have growth failure, delayed or incomplete sexual development with infertility, features associated with combined deficiencies of anterior pituitary hormones including growth hormone, TSH, LH, FSH, PrL, and ACTH. There are now two genes known to cause CPHD in humans - PIT1 and PROP1. While the number of families with reported PIT1 defects is small, preliminary studies indicate that the proportion of familial cases with PROP1 defects is significant. In this study we screened a series of affected individuals for mutations in the PROP1 gene. Here we report three new mutations found in the PROP1 genes of affected individuals but not in normal controls. Two of the mutations were identified in a pair of affected sibs who were both found to be compound heterozyotes with the maternal allele containing a C®T transition in the 16th codon and the paternal allele containing a G®A transition in codon 120. The C®T transition converts a CGA(Arg)® TGA(stop) codon terminating protein synthesis after amino acid 15. The G®A transition converts a CGC(Arg)®CAC(His) which corresponds to amino acid 52 in the third helix of the highly conserved homeodomain of PROP1. The third mutation was a heterozygous G®C transversion in codon 51 identified in two sporadic cases. This substitution converts a GGG(Gly)®GCG(Ala) and was not found in normal controls (0/30). From this study we conclude that heterogeneous defects in the PROP1 gene contribute to the ideology and spectrum of the CPHD phenotype observed in familial and sporadic CPHD subjects.

Program Nr: 785 from the 1999 ASHG Annual Meeting

Okihiro Syndrome, 3 new cases - is this an under diagnosed cause of deafness and 'squint'? *L.A. Colvin, M.I. Van Allen.* University of British Columbia, Vancouver, BC, Canada.

Okihiro (DR, MIM 126800) syndrome is a reportedly rare AD disorder with variable expression of sensorineural hearing loss, Duane's anomaly and thenar eminence hypoplasia. Only a half-dozen families and as many case reports are in the literature. Due to lack of awareness and subtle presentation of radial ray anomalies, we think Okihiro syndrome is an under diagnosed cause of deafness and 'squint'. Case A: 7 year old with Duane's anomaly, thenar hypoplasia and proximal placement of the thumb, conductive hearing loss, a submucous cleft and C3 and C4 fusion of lateral masses. She has a 46,XX, 22q.ish negative karyotype and a family history of a maternal grandfather with Duane's anomaly, and palatal cleft in a cousin. Case B: 3 and 6/12 year old with Duane's anomaly, thenar hypoplasia, truncal hypotonia, and plagiocephaly, normal development. She has 46,XX, karyotype and positive family history of 'squint' and lazy eyes. Case C: 7 year old female with Duane's anomaly, thenar hypoplasia, moderate hearing loss bilaterally, cleft palate, malar hypoplasia, mild micrognathia, microcephaly, developmental delay and vertebral abnormalities, but normal kidneys. She has a 46,XX, karyotype and family history mental retardation and isolated cleft lip. The variable presentation includes radial ray anomalies, ranging from thenar eminence hypoplasia with or without abnormally positioned thumbs, to absent thumbs and radius. Deafness is congenital and usually sensorineural. Less frequently reported anomalies are cervical vertebral anomalies, palatal clefts and renal anomalies. Okihiro syndrome overlaps with acro-renal-ocular syndrome. It is distinct from Fanconi syndrome, Holt-Oram syndrome, Wildervanck syndrome and 22q partial duplication and 22q11.2 deletion syndrome.

Program Nr: 786 from the 1999 ASHG Annual Meeting

BONE DYSPLASIAS AND IMPLICATIONS FOR GENETIC COUNSELING. *H.A. Cooper, J. Crowe, M.G. Butler*. Section of Medical Genetics and Molecular Medicine, The Children's Mercy Hospitals and Clinics, Kansas City, MO.

We report an 11-year-old male with a rare short stature syndrome. His height was 106.1 cm (-4 SD). He had a coarse appearing face with a depressed nasal bridge, short upturned nose, and mid-face hypoplasia. His intelligence was normal. Based on clinical evaluation at six years of age, the diagnosis of spondyloepiphyseal dysplasia (SED) was suspected. However, repeat genetic evaluation with radiologic studies at 11 years of age revealed delayed carpal ossification (-4 to 5 SD), striations of the metaphyses most notably in the distal femurs and the proximal tibias, lumbar lordosis, narrow interpedicular distance of the lumbar spine, and pear-shaped vertebral bodies with increased calcification of the concave surfaces. These findings are consistent with the diagnosis of SPONASTRIME (SPOndylar and NAsal changes, with STRiations of the MEtaphyses) dysplasia, and not SED.

SPONASTRIME dysplasia is a rare, autosomal recessive bone disorder first described by Fanconi et al. in 1983. Radiographic findings include abnormal vertebral bodies with age dependent changes, striations of the metaphyses, lumbar lordosis, scoliosis, and retarded ossification of the carpal bones. Physical features include severe short stature, midface hypoplasia, frontal bossing, and a saddle shaped nose. To date, 13 patients from seven families have been reported. Four additional patients have been reported with a variant of this condition, which includes mental retardation. Radiographic findings of SPONASTRIME dysplasia are distinguishable from SED, even though the physical appearance may be similar. The diagnosis of bone dysplasias can be difficult, as exemplified by this patient. Many bone dysplasias have overlapping radiographic findings and clinical presentation, but with different recurrence risks making genetic counseling a challenge.

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Program Nr: 787 from the 1999 ASHG Annual Meeting

Congenital diaphragmatic hernia in genetics. *K.P. Cusmano[1], B.G. Kousseff[2]*. 1) Pediatrics/Genetics, University of South Florida, Tampa, FL; 2) same.

Between 1-2-82 and 4-15-99 through the USF Genetic Clinics there were 93 probands with suspected or confirmed congenital diaphragmatic hernia (CDH) of the 35,414 probands/families evaluated. 50 were seen through the prenatal clinics. Among them were 5 in whom CDH was subsequently excluded; 3 had congenital cystic adenomatoid malformation of the lung, 1 had bronchogenic cyst, and 1 had trisomy 18. 24 survived and 26 expired including 3 miscarriages, 2 stillbirths, and 4 terminations. 34 had associated anomalies. Among these were 8 probands with Pentalogy of Cantrell, all deceased and previously reported [BDOAS 30:189-202, 1996]. The remaining 26 probands had in addition to CDH, a single defect, constellations of anomalies that were non-syndromic or syndromic CDH. The remaining 16 patients in the prenatal group had solitary CDH. 43 probands were evaluated through the pediatric genetic clinic. 17 survived and 26 died. 28 had associated abnormalities, 13 had solitary CDH. In this group there were 3 families each with first degree relatives having CDH. In one, sisters were affected; one of them had total anomalous pulmonary vein return. In the other, mother and daughter had solitary CDH. In the third, 2 brothers had CDH. This family most likely had autosomal dominant private syndrome with short stature, cleft palate, blepharophimosis, MVP, cryptorchidism, hypotonia and cardiomyopathy. Apart from the syndromic CDH there was no predilection towards a particular constellation of anomalies. Unique malformations included cyclopia, arhinia, proboscis, aphallia and blepharophimosis, all were with normal karyotypes. The syndromic CDH included 3 probands with Fryns syndrome and 1 each with Marfan, Saethre-Chotzen, Brachmann-DeLange, Ivemark, hypomelanosis of Ito, HARD, and limb-body wall syndromes. 9 patients had Pentalogy of Cantrell. CDH was found in 2 probands with trisomy 21 and in 1 each with trisomy 22 secondary to maternal translocation [11; 22], del 8p, mono 9p, del 15q and partial trisomy 1q. There were 3 maternal translocations, t [1; 9], t [11; 22] and t [7;13]. Despite having ECMO since 1993, in our experience CDH is still a high mortality genetically very heterogeneous condition without causative clues in solitary CDH.

Program Nr: 788 from the 1999 ASHG Annual Meeting

Christians Spondylo- digital syndrome: First sporadic male case.. *N.O. Dávalos[1,2], I.P. Dávalos[1,2], M.O. García-Cruz[3], Z. Nazará[4], D. García-Cruz[1,2]*. 1) Div. Genética, CIBO-IMSS, Guadalajara, México; 2) Doctorado en Genética Humana, CUCS-UdeG, Guadalajara, México; 3) Servicio de Labio y Paladar Hendido, HGR #46, IMSS, Guadalajara, México; 4) Servicio de Radiología, HE-CMNO, IMSS, Guadalajara, México.

The Christians spondylo-digital syndrome has been previously delineated in two familial cases (mother and daughter affected in both), it is characterized by metacarpal and metatarsal asymmetry, tarsal and carpal fusions, syndactyly, articular dysplasia and platyspondyly. The purpose of this paper is to describe the first sporadic male case in the literature. CASE REPORT: The propositus was 16 years old. Since birth unilateral cryptorchidism and digital malformations were detected. He also showed psychomotor delayed. Clinically he showed on the left hand: distal flexion contractures of 2nd 5th fingers with wide knucles and radial deviation of the distal phalanx of the 5th finger; radial deviation of 4th and 5th fingers, ulnar deviation of 2nd finger and prominent metacarpophalangeal joints. On the right hand: distal flexion contractures of 2nd, 4th and 5th fingers, wide knucles of 2nd, 4th and 5th fingers, ulnar deviation of 2nd and 3rd fingers, prominent metacarpophalangeal joints, bilateral cutaneous syndactyly, absence of distal interphalangeal creases, and thenar and hypothenar hypoplasia. Bilateral hallux valgus and shortened 2nd-5th fingers in both feet. He also presented oral anomalies: mandibular prognathism, permanent dentition with dental malposition and crowding, and a high-arched V-shaped palate. The routine laboratory examination, and karyotype yielded normal or negative results.The cardiologic and EKG evaluation were normal. Since the 2 previous familial reported cases were females, an autosomal dominant inheritance could be more feasible than a dominant X-linked inheritance, mainly by the presence of moderate clinical features noticed in the present case. On the other hand, the fact that female cases are more severely affected than the present male case, probably could be explained by maternal genomic imprinting. Althougth more cases must be studied to confirm this hypothesis.

Program Nr: 789 from the 1999 ASHG Annual Meeting

New families referred for confirmation of diagnosis of Marfan syndrome: the experience of a multidisciplinary approach in Wales. *S.J. Davies[1], M. Nicol[1], V. Booker[2], D. Wilson[2], A.G. Stuart[2].* 1) Inst Medical Genetics, Univ Hosp Wales, Cardiff, Wales; 2) Congenital Heart Unit, University Hospital of Wales, Cardiff, Wales.

New referrals to a multidisciplinary team for confirmation of diagnosis of Marfan syndrome (MFS) were reviewed for the three years from 1st of March 1995. Investigation included a full family history, reviewing clinical notes with consent, clinical examination, cardiological evaluation and ophthalmologic assessment. Eighty-eight new families were referred. The probands were 58 males and 30 females aged between 1 and 75 years. 162 individuals were evaluated according to the criteria of the Ghent nosology. Seventeen families had been referred with a definite diagnosis of Marfan syndrome (MFS), ten families had a family history of a first degree relative with diagnosed MFS and sixty-one families were referred with a queried diagnosis of MFS. Following investigation, three of the seventeen families with a definite diagnosis were reassigned as unaffected as they did not fulfil the diagnostic requirements. The ten probands referred with a 50% risk were all unaffected and in one family the diagnosis was incorrect on the supposed affected parent. Five families were diagnosed as having a condition other than MFS, namely contractural arachnodactyly, annulo aortic ectasia, Ehlers- Danlos Type IV, Stickler syndrome and dominant ectopia lentis. Eight new families were diagnosed as having MFS. These included 14 affected individuals, 7 adults and 7 children. Three probands had other affected family members whilst five were sporadic. Only one of these newly diagnosed families had a history of MFS cardiac related deaths. Overall 22 families were confirmed or newly diagnosed as MFS (25%). In 17 families the diagnosis was changed (19%). In 57 families the diagnosis could be refuted following careful assessment (64%). This review emphasises the importance of a multidisciplinary approach to the diagnosis of Marfan syndrome where a correct diagnosis is essential to prevent major complications. Furthermore, an incorrect diagnosis leads to unnecessary screening and anxiety about health, insurance etc.

Program Nr: 790 from the 1999 ASHG Annual Meeting

First report of two siblings with apparently isolated odontohypophosphatasia: Dominant or recessive inheritance? *A.B. Decker*[1], *C.T. Coccia*[2], *G.L. Feldman*[1]. 1) Medical Genetics, Henry Ford Hospital, Detroit, MI; 2) Pediatric Dentistry, Henry Ford Hospital, Detroit, MI.

Hypophosphatasia is characterized by reduced activity of tissue non-specific alkaline phosphatase (TNSALP), elevated serum pyridoxal 5'-phosphate (PLP) concentration, and increased urinary phosphoethanolamine (PEA) levels. Hypophosphatasia is heterogeneous, ranging from neonatal death caused by profound skeletal hypomineralization, to premature loss of primary teeth. Odontohypophosphatasia describes dental abnormalities and biochemical evidence of hypophosphatasia, with no evidence of bone involvement. Odontohypophosphatasia is often considered within the spectrum of hypophophatasia. Dominant and recessive inheritance have been described for odontohypophosphatasia. We report two siblings with premature loosening and loss of primary incisors (ages 24 and 26 months), low TNSALP levels and elevated PLP levels. PEA analysis has not yet been performed. Radiographic studies of the hands and knees of both sibs were normal, and there is no history of fractures, bowing or joint pain. The parents have no history of premature loss of teeth, other dental, bone or joint symptoms. However, the father has decreased TNSALP levels and elevated PLP levels, suggestive of biochemical evidence of TNSALP gene expression. The mother has serum TNSALP levels in the lower normal range, and normal PLP levels. Hypophosphatasia may result from two abnormal alleles which alone or in combination may cause the disease. One abnormal allele may function in an autosomal dominant manner, resulting in a mild phenotype. If two abnormal alleles are present, a more severe phenotype may result. The TSNALP gene has been localized to chromosome 1 (1p34-36.1), and over 70 mutations have been described. This family may represent autosomal dominant inheritance, with variable expressivity in the father, or autosomal recessive inheritance. Mutation analysis is in progress for this family. Although studies have found specific mutations and deletions associated with the various forms of this condition, genotype-phenotype studies are needed to learn more about the changes in the TNSALP gene and the mode of inheritance.

Program Nr: 791 from the 1999 ASHG Annual Meeting

A novel disorder of Pena-Shokeir phenotype and pachygyria due to autosomal recessive inheritance. *K.M. Dent[1], C.E. Miller[2], J.C. Carey[1], C.O. Leonard[1]*. 1) Pediatrics, University of Utah, Salt Lake City, UT; 2) Ob/Gyn, University of Utah, Salt Lake City, UT.

The Pena-Shokeir phenotype or fetal akinesia/hypokinesia sequence is a rare, lethal disorder characterized by multiple joint contractures, pulmonary hypoplasia, CNS dysfunction and distinctive facial anomalies including hypertelorism, low-set malformed ears, micrognathia, and depressed nasal tip. The etiology of the Pena-Shokeir phenotype is heterogeneous and no specific cause or pathogenesis has been identified in a substantial portion of cases. This is due, in part, to the difficulty of defining discrete subsets of children with the Pena-Shokeir phenotype that are etiologically related to one another. For example, severe joint contractures are characteristic of many disorders (e.g., cerebro-oculo-facio skeletal syndrome, lethal multiple pterygium syndrome, and Neu-Laxova syndrome) that result in diminished fetal movement. We describe a single family with three female siblings born to non-consanguineous parents sharing a unique constellation of features that define a discrete subset of children with the Pena-Shokeir phenotype. In addition to multiple congenital contractures and pulmonary hypoplasia, these infants had varying combinations of pachygyria, adrenal hypoplasia, hypertelorism, cleft palate, short neck, overlapping fingers, and varus deformities with rocker bottom foot. Polyhydramnios was observed in all three pregnancies. The third pregnancy was diagnosed prenatally due to polyhydramnios, contractures, clubfoot, and decreased fetal movement. All died shortly after birth due to respiratory insufficiency. Chromosome analysis (including FISH for 17p in one patient) was normal in all three cases. While pachygyria has been described in cases with congenital contractures, the phenotype of these children is different. This condition likely represents a new, probably autosomal recessive disorder.

Program Nr: 792 from the 1999 ASHG Annual Meeting

A new disorder or a variant form of Chanarin-Dorfman syndrome in twin sisters. *V.M. Der Kaloustian[1], J. Davignon[2], C. McCuaig[3], T. Babineau[1], B. Moroz[4], J.P. Roman[5], S. Melançon[5].* 1) The F. Clarke Fraser Clinical Genetics Unit, Departments of Pediatrics and Human Genetics, Montreal Children's Hospital and McGill University, Montreal, Quebec, Canada;; 2) Clinical Research Institute of Montreal, Montreal, Quebec, Canada;; 3) Department of Dermatology, Ste-Justine Hospital and Université de Montréal, Québec, Canada;; 4) Department of Dermatology, Montreal Children's Hospital and McGill University, Montreal, Quebec, Canada;; 5) Department of Medicine, CHUM, Université de Montréal, Montréal, Québec, Canada and Procréa, Services Génétiques, Montréal, Québec, Canada.

We present identical twin sisters with thick, yellow-brown, greasy and hyperkeratotic lesions of the face, scalp, and various parts of the trunk and extremities. The onset of the condition was at the age of 16 years in one of them and 27 years in the other. The clinical picture also includes Raynaud phenomenon and autoimmune thyroid disease. Mitral regurgitation is present in one of the sisters and scoliosis in the other. The eyes and intelligence are normal.

Histology of the skin reveals hyperkeratosis and a marked hypertrophy of the sebaceous glands, with small lipid vacuoles in the cytoplasms of the epidermal basal cells. Electron microscopy confirmed the intracytoplasmic location of the lipid vacuoles, without a surroundiing membrane, in the cells of the basal cell layer. These findings favour the diagnosis of the Chanarin-Dorfman syndrome.

Lipoprotein electrophoresis reveals a more rapid migration towards the anode. Cholesterol esters are found in the tears. The blood levels of retinol are very low. An excess of oxoproline (pyroglutamic acid) is present in the cutaneous lipid exudate with normal urine levels of all organic acids.

In spite of a strong suggestion of the Chanarin-Dorfman syndrome by skin biopsy, we believe that our patients have many clinical and laboratory differences, pointing to a variant form of this syndrome or a completely new entity.

Program Nr: 793 from the 1999 ASHG Annual Meeting

Macrocephaly in autism pedigrees. *C.K. Deutsch[1,2], S. Folstein[1,3], H. Tager-Flusberg[1,4], K.G. Gordon-Vaughn[1,3].* 1) Psychobiology Program, Eunice Kennedy Shriver Ctr, Waltham, MA; 2) Harvard Medical School, Boston, MA; 3) New England Medical Center, Boston, MA; 4) University of Massachusetts, Boston, MA.

A number of laboratories have found increased brain volume and macrocephaly to be statistically overrepresented in autism. Is this cranial enlargement uniform, or during development are some brain and cranial vault regions disproportionately affected? We have used objective, quantitative measures of craniofacial dysmorphology to assess cranial dimensions in a sample of 108 autistic probands. We have confirmed a statistical excess of increased head circumference in autism (25.0%; macrocephaly defined as a z-score greater than 1.5), exceeding the population baserate of 6.7% ($p < .01$). Goodness-of-fit tests reveal that the circumference scores in the autistic group are shifted in the direction of higher scores relative to the general population, yet a Gaussian distribution is still maintained. There was a marked increase in cranial width measured between the left and right eurion ($p < .01$). In contrast, the cranial length (the anterior-posterior linear distance between the glabella and opisthocranion) was normal, creating a brachycephalic pattern. Further, the presence of macrocephaly and increased biparietal width are predictive of the autistic probands' behavioral phenotype. The subgroup of patients with enlarged cranial dimensions is particularly intellectually impaired in comparison to autistic individuals with normal head size, with a specific impact on language skills. The dysmorphic phenotype described above for autistic probands was also seen among their first-degree relatives, even among those who did not meet criteria for autism ($N = 50$). The rate of macrocephaly among parents ($N = 39$) was 15.8% ($p < .05$) and among non-autistic siblings ($N = 11$) was 36.4% ($p < .01$). The pattern of excessive cranial width but not length was maintained among relatives ($p < .05$ and n.s., respectively). These data imply a specificity of brain growth patterns in autistic macrocephaly that is conserved among relatives. The implications of these findings for models of brain maldevelopment are also discussed. (This research was supported by NIH PO1 DC03610, R01 MH55135, and P30 HD04147.).

Program Nr: 794 from the 1999 ASHG Annual Meeting

Hypothyroidism in an infant with ectodermal dysplasia and cleft lip and palate. *J. DiMartino-Nardi[1], A. Palomba[2], A. Shanske[1]*. 1) Pediatrics, Montefiore Medical Center, Bronx, NY; 2) Pediatrics, Bronx Municipal Hospital Center, Bronx, NY.

The association of ectodermal dysplasia (ED) and clefting deformity is well known and has been reported in patients with a variety of disorders.A combination of oral clefting, ectropion and ED are the cardinal features of the blepharocheilodontic syndrome.Reports of ED and hypothyroidism are rare.We recently evaluated an infant with ED, cleft lip and palate and primary hypothyroidism.

AK was the 3.8 kg product of an uneventful pregnancy born to consanguineous Pakistani parents with a left-sided cleft lip and palate,ectropion,and dermoid cysts on the face and scalp.He was admitted at 4 months of age to our hospital because of respiratory distress.His physical examination at that time revealed a malnourished hypotonic infant with the additional findings of ankyloglossia, a large fontanelle, sparse lashes, absent eyebrows and normal nails. His length was 59 cm, weight 4 kg, and HC was 38.5 cm.The anterior fontanelle measured 5 by 4.5 cm and the primary dentition had not yet erupted. Imaging studies revealed a pericardial effusion, dilated loops of bowel, the bone age of a newborn, the absence of tooth follicles, and a normal MRI of the head.Laboratory investigation revealed a normal male karyotype,a TSH greater than 700mU/ml,a barely detectable level of T_4,and a normal cortisol.He was begun on synthroid therapy with resolution of the pericardial effusion and slow weight gain.

The association of ED and clefting is a rare event and when reported usually includes nail dysplasia.Our patient has normal nails,dermoid cysts,ectropion,and anodontia as well as hypothyroidism.It is difficult to implicate the action of a teratogen because of the variability of the critical times of origin and the involvement of derivatives of 2 germ layers.However, consanguinity suggests homozygosity for a single pleiotropic recessive gene.In fact, Zadik (Clin Dysmorph 1:24-27, 1983) reported a case with dermoid cysts,hyothyroidism,cleft palate and hypodontia.We propose that this and our case represent a discrete disorder.

Program Nr: 795 from the 1999 ASHG Annual Meeting

Female with autistic disorder and monosomy X (Turner syndrome): parent-of-origin effect of the X chromosome.

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Skuse et al. (*Nature*, 1997) had previously reported three patients with the co-occurrence of Autistic Disorder (AD) and monosomy X (Turner syndrome) as part of a study examining social cognition and parent-of-origin effect of the X chromosome. They concluded that monosomy X individuals with a maternally-derived X chromosome exhibited more deficits in social cognition and verbal I.Q. than individuals with a paternally-derived X chromosome. We have independently ascertained and examined a fourth documented patient with idiopathic AD and monosomy X. The patient met DSM-IV/ICD-10 criteria for AD verified by the Autism Diagnostic Interview Revised (ADI-R) and exhibited both social and verbal deficits. Based on the findings of Skuse et al., we conducted parent-of-origin studies in our proband by genotyping X chromosome markers in the patient and her parents. We found that the patient's X chromosome was of maternal origin. These findings provide further support for the hypothesis that parent-of-origin of the X chromosome influences social cognition and could provide insight into the preponderance of males with AD.

Program Nr: 796 from the 1999 ASHG Annual Meeting

A new association comprising congenital hydrocephalus, albinism, megalocornea, retinal coloboma, and cryptorchidism. *P. Dubé[1], V.M. Der Kaloustian[2], R.K. Koenekoop[1]*. 1) Department of Ophthalmology; 2) Departments of Pediatrics and Human Genetics, McGill University, Montréal, Québec, Canada.

We present a 5 year-old-boy with congenital hydrocephalus, prominent metopic ridge with trigonocephaly, albinism, megalocornea, retinal coloboma, bilateral epicanthic folds, depressed nasal bridge, micrognathia, low-set ears, cryptorchidism, developmental retardation and generalized hypotonia. The clinical presentation resembles the deletion 9p syndrome. However, the karyotype is normal 46,XY. Certain features such as congenital hydrocephalus, albinism, megalocornea, and retinal coloboma have not been reported as part of the deletion 9p syndrome. We therefore suggest that this is a new association that has not been described previously in the literature.

Program Nr: 797 from the 1999 ASHG Annual Meeting

Acrocallosal syndrome with MRI findings of perisylvian syndrome: expansion of the phenotype. *S.A.K. Durrani*[1], *C.J. Tifft*[2]. 1) Medical Genetics Branch, National Human Genome Research Institute, NIH, Bethesda, MD; 2) Department of Medical Genetics, Childrens National Medical Center, Washington D.C.

Acrocallosal syndrome is an autosomal recessive disorder featuring postaxial polydactyly, limb anomalies and an absent corpus callosum. Here we describe a patient with the additional features of perisylvian changes on MRI, median cleft lip and bronchial anomalies.

The patient is a 13 year old 46,XY male who presented at birth with a midline cleft lip and multiple limb anomalies. The past medical history is also notable for multiple bronchial stenoses and tracheomalacia, and a slipped capital femoral epiphysis. He has cognitive deficits and significant motor and language delays. The family history is significant for a distant maternal relative with syndactyly of hands and toes.

The child is macrocephalic and brachycephalic with a short narrow forehead and palpable metopic ridge. There is synophrys and bilateral epicanthal folds. The nose has a broad alar base and bulbous tip. The major helices are incomplete and the lobules large. A repaired midline cleft lip with linear striae along both nasolabial folds and preauricular regions are noted. The left thumb is broad, short and medially deviated. The right hand has medial deviation of the third and lateral deviation of the fourth fingers. The fifth finger is hypoplastic and duplicated. The radii are long bilaterally preventing full extension and pronation. The child is moderately obsese. An MRI demonstrates bilateral perisylvian hypoplasia, a hypoplastic corpus callosum and a posterior fossa arachnoid cyst.

Acrocallosal syndrome has a broad clinical presentation including macrocephaly, hypertelorism, polydactyly, limb anomalies, arachnoid cyst, occipital prominence and dysplastic corpus callosum as seen in our patient. MRI changes suggestive of the perisylvian syndrome have not been previously reported. This case may represent a widened clinical spectrum for the disorder.

Program Nr: 798 from the 1999 ASHG Annual Meeting

Craniosynostosis in Prader-Willi syndrome: association or coincidence? *S. Dyack[1], M. Sgro[2], I. Teshima[3], P. Ray[3], L. Steele[3], A. Lowinsky[2], D. Chitayat[1,4].* 1) Div. Clinical & Metabolic Genetics, Hosp for Sick Children, University of Toronto, Toronto, ON, Canada; 2) Dept. Obstetrics/Gynecology, University Health Network, UofT, Toronto, ON, Canada; 3) Pediatric Lab Medicine, Hosp for Sick Children, UofT, Toronto, ON, Canada; 4) The Prenatal Diagnosis Program, UofT, Toronto, ON, Canada.

Prader-Willi syndrome (PWS) is a genetic condition most commonly associated with a deletion of 15q11-13 on the paternal chromosome. Affected patients present in the neonatal period with hypotonia, feeding difficulties, failure to thrive, and characteristic facial features. Craniosynostosis has not been reported in association with this condition. We report a patient with Prader-Willi syndrome and craniosynostosis. The patient was born to a healty 34 year-old G5P4SA1L4 woman of Pakistani descent, and a 36 year-old healthy father of the same descent. The parents were nonconsanguineous. There was very little antenatal care prior to the third trimester. Ultrasounds performed at approximately 32 and 36 weeks gestation revealed a breech position, and no fetal anomalies. The mother reported poor fetal movements throughout the pregnancy. The baby was delivered vaginally at term after induction of labor. Her birth weight was 3180 grams and the Apgar scores were 7 and 8 at 1 and 5 minutes respectively. Severe hypotonia and dysmorphic features were noted at birth, including an abnormal head shape. A skull x-ray and a 3D CT scan showed bicoronal suture craniosynostosis. Chromosome analysis was 46,XX and FISH analysis using probes for SNRPN and D15S10 showed a deletion of 15q11-13. DNA analysis for FGFR2 and 3 was performed and no mutation was detected. Review of the literature revealed 1 case of PWS with craniosynostosis; however, neither x-rays nor CT scan were done to confirm the craniosynostosis.

In summary, this is the first case of PWS and confirmed craniosynostosis. We speculate that the deletion of 15q11-13 resulted in a deletion of a dominant gene or genes associated with craniosynostosis.

Program Nr: 799 from the 1999 ASHG Annual Meeting

Progressive erosive arthropathy, characteristic non-coarse facies, malar erythema, facial telangiectasia, and multiple Nevi: A new syndrome. *A.M. Elliott[1], E.M. Azouz[2], A.S. Teebi[1]*. 1) Depts Peds & Human Genetics, MCH & McGill Univ, Montreal, PQ, Canada; 2) Deptartment of Diagnostic Imaging, MCH & McGill Univ, Montreal, PQ, Canada.

Syndromic forms of progressive arthropathy are extremely rare. They are either associated with significant epiphyseal and/or vertebral involvement suggestive of a skeletal dysplasia or with multisystem involvement suggestive of a storage disorder or a disorder of proteins of the extracellular matrix. We report a 27-year-old man with an apparently new syndromic form of progressive erosive arthropathy and contractures of small and large joints associated with mild epiphyseal changes, normal vertebrae and generalized osteopenia. The patient has a characteristic craniofacial appearance, dermatological abnormalities, and normal intelligence. The head is large with frontal bossing; the face is elongated with malar hypoplasia, thin upper lip, prominent lower jaw, high arched palate, dental malocclusion, and prominent ears with absent ear lobules. Dermatological abnormalities include malar erythema and facial telangiectasia together with multiple nevi and lentigens all over the body. Pseudorheumatoid arthropathy, spondyloathropathy, and Borrone dermatocardioskeletal syndrome (Am J Med Genet 46: 228-234, 1993) were considered in the differential diagnosis and were excluded. Also, no similar cases have been found in POSSUM or London Dysmorphology databases.

Program Nr: 800 from the 1999 ASHG Annual Meeting

Progressive Encephalopathy-Edema-Hypsarrhythmia-Optic atrophy (PEHO) syndrome. *G.M. Enns[1], A. Plump[2], C.S. Hoyt[3], E. Sherr[4], V.A. Cox[2], M. Golabi[2]*. 1) Div. Med. Genet., Stanford Univ.,CA; 2) Div. Med. Genet., U.C.S.F., CA; 3) Dept. Ophthal., U.C.S.F., CA; 4) Div. Ped. Neurol., U.C.S.F., CA.

PEHO syndrome is an autosomal recessive infantile encephalopathy of unknown origin. Typical findings start at age 2 weeks to 3 months and include postnatal microcephaly, severe hypotonia, hyperreflexia, infantile spasms, and extremity edema. Facial features are characterized by a narrow forehead, epicanthic folds, puffy cheeks, midface hypoplasia, protruding earlobes, high-arched palate, and open mouth. A small cerebellum and/or brainstem are often present. PEHO syndrome has been primarily described in the Finnish population, aside from two case reports in single Japanese and French Canadian families. We present two patients with different ethnic backgrounds with PEHO syndrome. Patient 1 is a 17 month old boy of mixed European descent born to non-consanguineous parents. A lack of visual tracking, hypotonia, and hypsarrhythmia were noted in infancy. Postnatal microcephaly, optic atrophy, characteristic facial features, tapering digits, and peripheral edema were also present. A head MRI at 9 months showed gray matter heterotopias and hypoplasia of the corpus callosum, pituitary, and pons. Metabolic labs and karyotype were normal. Patient 2 is a 3 year old Hispanic girl born to consanguineous parents. Clinical findings similar to patient 1 were present in infancy. A head MRI at 4 months showed delayed myelination. Metabolic labs, including lymphocyte EM studies, and karyotype were normal. A muscle biopsy showed fiber type disproportion with atrophy of type I fibers and hypertrophy of type II fibers. Muscle mitochondrial respiratory chain analysis was normal. The clinical course has been progressive with loss of all milestones. We conclude that PEHO syndrome may be present in diverse ethnic groups. Furthermore, gray matter heterotopias, pituitary hypoplasia, and muscle fiber type disproportion may occur in PEHO syndrome. Because of the significant implications for prognostic and genetic counseling, patients with infantile spasms and progressive loss of milestones should be evaluated for the characteristic features of PEHO syndrome.

Program Nr: 801 from the 1999 ASHG Annual Meeting

Liver fibrocystic disease and polydactyly in two unrelated patients: New syndromatic entity? *C. Esmer[1], E. Lieberman[1], A. Alvarez[2], F. Zarate[3], V. Del Castillo[1]*. 1) Department of Research in Human Genetics; 2) Patology Department; 3) Gastroenterology Department. National Institute of Pediatrics, DF, Mexico.

Liver fibrocystic disease (LFCD) consisting on dilatation of the intrahepatic bile ducts accompanied by variable degrees of fibrosis can be present alone or be found as part of many syndromes. There are at least 37 conditions, among which are metabolic disorders, bone dysplasias and other mendelian and sporadic diseases. We present two unrelated patients with LFCD and polydactyly. Both abnormalities have been described for four genetic conditions: Bardet-Biedl syndrome, Meckel syndrome, Jeune's asphyxiating thoracic dysplasia and Fraser-Jaquier-Chen syndrome. Bardet-Biedl Syndrome (BBS) is characterized by autosomal recessive inheritance, pigmentary retinopathy, obesity, polydactyly, mental retardation and hypogonadism. The existence of BBS and liver abnormalities has been previously described. Case 1 is a mentally retarded boy 18 months of age, he has hands and feet hexadactyly, kidney failure, convergent strabismus and liver fibrosis, as criteria for suspecting BBS. Case 2 is a 1 year-old child of consanguineous parents, with liver fibrosis and polydactyly in both hands and feet without any other cardinal data supporting BBS. Difficulties in the early diagnosis of BBS have been previously reported for the disease and this is explained by the clinical heterogeneity of manifestations and by the variability at the ages that they appear. Definitively, our patients didn't meet all the criteria to conclude BBS as diagnosis and a new genetic syndrome with an autosomal recessive inheritance must be considered as the cause of their abnormalities.

Program Nr: 802 from the 1999 ASHG Annual Meeting

Hydranencephaly and severe rib anomalies: an unusual presentation of Seckel syndrome. *J.A. Evans[1,2], B.N. Chodirker[1,2], S.R. Sanders[1,2], C.R. Greenberg[1,2]*. 1) University of Manitoba, Winnipeg, MB; 2) Children's Hospital, Winnipeg, MB, Canada.

Seckel syndrome, characterized by severe intrauterine and postnatal growth retardation, marked microcephaly, a "bird-headed" facial appearance and mental retardation has proved to be much rarer than initially thought. We report an infant with classic Seckel syndrome in whom hydranencephaly, severe rib defects and other anomalies were also present.

The female proband was stillborn at term to a 21-year-old G2P1 woman. Delivery was by cesarean section for abruptio placentae. The mother had been followed since 17 weeks gestation when growth retardation and an unusual skull shape were first noted on ultrasonographic examination. Growth parameters at birth were: weight 1.1kg, length 36cm and head circumference 24cm (all <<5th centile). Additional anomalies suggesting Seckel syndrome were sloping forehead, convex beaked nose, marked micrognathia, dysplastic ears, high-arched palate with a central membraneous defect, bilateral fifth finger clinodactyly with a single digital crease, delayed dyssynchronous ossification and fibular hypoplasia. Hydranencephaly, corneal clouding, non-fixation of the colon and dysplastic cartilagineous renal foci were also seen. The right clavicle, sternum, all ribs other than those at T8-11, the pubic rami and the tali were unossified. A subsequent pregnancy of the mother was terminated because of severe microcephaly, suggesting a recurrence.

Although hydranencephaly has not been reported previously in Seckel syndrome, Sugio et al. (1993) described a boy with cerebral and cerebellar hypoplasia leading to an essentially empty intracranial space on CT scan, and several other cases have had less severe dysgenetic anomalies such as lissencephaly, pachygyria and cerebral cysts. Brain defects also occur in other primordial dwarfisms. However, the lack of a more generalized osteodysplasia and the occasional occurrence of visceral anomalies (e.g. renal defects, tracheoesophageal fistula) in Seckel syndrome are further evidence that it is a pathogenetically distinct entity.

Program Nr: 803 from the 1999 ASHG Annual Meeting

Queen Of Punt Syndrome? *T.I. Farag[1], M.A. Sabry[2], A. Iskandar[3]*. 1) Dalhousie University, Halifax, NS, Canada; 2) Harvard University, Boston, Massachusetts, USA; 3) Purdue University, Hammond, Indiana, USA.

Critical bioanthropological and medico-genetic dissection of ancient art and mummies is a fascinating emerging discipline. In Cairo Museum, a relief (#11465) from Queen Hatshepsut's tomb (c.1479-1457 B.C.) in Deir el-Bahari shows the Queen of Punt(Somalia) with a lined face, hyperlordosis, corpulent body, gluteofemoral obesity and apparent symmetrically distributed hamarto-neoplastic swellings on the trunk, arms and thighs, sparing her face, neck, hands and feet. The portrait of her daughter, on the same relief, shows a similar posture. In the absence of the Queen's mummy (died nearly 34 centuries ago), there is insufficient substance, too much speculation and too few facts to decide the precise diagnosis.

Here we review different hypotheses regarding the Queen's characteristic phenotype including artistic pseudopathology, racial steatopygy, filariasis, myxodema, rickets, bilateral congenital hip dislocations in addition to the following 8 monogenic disorders: Achondroplasia, Dercum disease, lipodystrophy, Launois-Bensaude syndrome, Neurofibromatosis, familial obesity, Proteus syndrome and X-linked dominant hypophosphatemic rickets (MIM 100800, 103200, 151660, 151800, 162200, 164160, 176920, 307800).

It seems that the Queen's portrait represents a truly unique phenotype which, if accepted as a new entity, can be coined as Queen of Punt Syndrome. Syndromologists are invited to contribute their expertise to solve the puzzle and help in the precise delineation of the Queen' s alleged pathological obesity and lumbar hyperlordosis.

Acknowledgments: Thanks to Prof. John M. Opitz and Prof. R.Neil Schimke for their valuable comments and contributions.

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Program Nr: 804 from the 1999 ASHG Annual Meeting

A Locus for Brooke-Spiegler Syndrome assigned to 16q12-q13. *C.D Fenske[1], P. Banerjee[2], C.A. Holden[2], N.D. Carter[1]*. 1) Medical Genetics Unit, St. George's Hospital Medical School , London, UK; 2) St. Helier's Hospital, Carshalton, Surrey, UK.

Brooke-Spiegler syndrome (BSS) is an autosomal dominantly inherited disease, characterised by the development of multiple trichoepitheliomas and cylindromas. Spiradenomas and basal cell carcinomas have also been observed in this disease. The gene for familial cylindromatosis has been localised to chromosome 16q12-q13 and loss of heterozygosity suggests that the gene involved is a tumour suppressor. The gene for multiple familial trichoepithelioma has been localised to chromosome 9p21 with a second form associated with cylindromas, determined by a gene on 16q. Heterogeneity of symptoms and tumour phenotype are found in families affected with BSS; however, familial cylindromas are invariably accompanied by trichoepitheliomas. DNA extracted from individuals of a family with three members affected with BSS was amplified using primer pairs in the regions of chromosomes 16 and 9 (candidate regions for familial cylindromatosis and trichoepithelioma respectively). Results identifying the segregation of haplotypes through the family eliminated 9p21 as a candidate region for a BSS locus. However, there was strong segregation with BSS affected members of the family of genotypes obtained with the markers on 16q12-q13. We conclude, therefore, that this area of the genome contains a locus for Brooke-Spiegler Syndrome. No obvious candidate genes lie within this region, but cDNA has been characterised at one locus (Cda01g10) where the base sequence is highly similar to a negative regulator of transcription subunit 1 in Saccharomyces cerevisiae. Given the frequent heterogeneity within families, other loci may be involved.

Program Nr: 805 from the 1999 ASHG Annual Meeting

A Clinical Genetic Study of Children Attending Special Schools (for Learning Disability) in Edinburgh. *D.R. FitzPatrick[1], P. Pearson[1], S. Halpin[2], P. Jackson[2].* 1) Human Genetics Unit, Edinburgh University, Edinburgh, Scotland; 2) Community Child Health, Sick Childrens Hospital, Edinburgh.

We wished to assess the completeness of genetic investigation in a community-based population of children with mild to moderate learning disability (LD). We performed a clinical genetic study of children attending two state-funded special schools in Edinburgh, one educates children of ages 5-12 years and the other 13-18 years. Permission was obtained from parents or guardians to review the records (school medical, community pediatric, laboratory and hospital) and clinically examine 105 (82%) children within the school setting. The sex ratio of the group was 1.62 (m:f 65:40). 20 children (19%) had previously identified causes for their disability (5 autosomal dominant disorders, 2 autosomal recessive, 2 X-linked recessive, 7 chromosomal, 3 neonatal infections and 1 malformation syndrome). Of the remaining 85 children (termed learning disability-cause unknown LDCU), chromosome analysis had been performed in 18/85 (21.2%) cases and fragile X status in 19/85 (22.4%), only 5/85 (5.9%) had both investigations recorded. Among the LDCU group 43/85 (50.6%) had a positive family history. 30 (35.3%) had at least one first degree relative with LD (15 with affected sibling(s), 11with affected parent(s), 4 with affected parent(s) and sibling(s)) and a further 13 had a second or third degree relative with LD. There is a highly significant genetic contribution to the etiology of LDCU in this population with relatively poor levels of genetic investigation. It is likely that much of difficulty in achieving adequate levels of care for these family relate to social circumstances rather than failure of referral to specialist services. The need for a community-based approach to investigation of LD is obvious.

Program Nr: 806 from the 1999 ASHG Annual Meeting

Clinical Features of Four Males and an Obligate Carrier in a Family with Lenz Syndrome. *S. Forrester[1], S. Rothert[1], R. Urban[2], M. Kovach[1], V. Kimonis[1]*. 1) Department of Pediatrics, SIU School of Medicine, Springfield, IL; 2) Department of Ophthalmology, Tampa General Hospital, Tampa, FL.

Lenz syndrome is a rare X-linked recessive syndrome first described by Lenz in 1955. Clinical features include anophthalmos or microphthalmos, microcephaly, mental retardation, external ear, digital, cardiac, skeletal, and urogenital anomalies (Traboulsi, et al 1988). Graham, et al (1991) localized the gene to Xq27-Xq28 in a Northern Ireland family with X-linked anophthalmos. However, the affected members in this family lacked digital, cardiac, and urogenital anomalies typically seen in Lenz syndrome. We present three brothers (ages 15 years, 9 years, and 18 months) and a maternal uncle (age 27 years) with Lenz syndrome. All four males have congenital anophthalmos with absence of the globes, optic nerve, and optic chiasm, and the youngest male has unilateral microphthalmos with corneal sclerosis. Delayed milestones and moderate to severe mental retardation were evident in the four males, and three of them have behavior problems. On physical examination, a high arched palate, pectus excavatum, finger and toe syndactyly and clinodactyly, fetal pads on all fingers and toes, and decreased muscle tone were noted in all four males. Scoliosis and outer ear anomalies were noted in three of the four males. The oldest brother has dilated left atrium of the heart and duplicated renal system, and the maternal uncle has right ventricular hypertrophy. An obligate carrier was noted to have syndactyly of the 2-3rdtoes bilaterally and dysplastic ears. We believe this family will add to the spectrum of clinical features of Lenz syndrome. Many of the cases published to date for Lenz syndrome are isolated reports. Linkage analysis in this family is in progress to identify the gene responsible for Lenz syndrome.

Program Nr: 807 from the 1999 ASHG Annual Meeting

Novel presentation of Goldenhar syndrome associated with tracheobronchial cartilaginous nodules in a premature neonate. *B.C. Freitag[1], H. Milczuk[1,2], M. Grompe[1,3], D.M. Pillers[1,3].* 1) Pediatrics, Oregon Health Sciences Univ., Portland, OR; 2) Otolaryngology and Head and Neck Surgery, Oregon Health Sciences Univ., Portland, OR; 3) Molecular and Medical Genetics, Oregon Health Sciences Univ., Portland, OR.

Goldenhar syndrome represents one end of a spectrum of disorders known as Oculoauriculovertebral syndrome (OAVS). While there is no agreement on minimal diagnostic criteria, most patients have abnormalities of the first and second branchial arch derivatives, including but not limited to microtia, mandibular hypoplasia, hemifacial microsomia, and ocular anomalies such as epibulbar dermoids and microphthalmia, as well as vertebral anomalies. Other manifestations include renal, cardiac, and radial limb defects, and rarely tracheoesophageal (TE) anomalies. Previously described tracheoesophageal malformations include TE fistula with or without esophageal atresia, tracheal cleft, and one report of vertically fused tracheobronchial cartilage. We report a patient with clinical features consistent with Goldenhar syndrome. The proband was a 28 week EGA premature male infant transferred to OHSU for tracheostomy placement secondary to inability to protect his airway. Diagnostic laryngobronchoscopy identified cartilaginous nodules of the trachea and right mainstem bronchus, a novel finding associated with this disorder. Other physical findings included low-set malformed auricles, micrognathia, epibulbar dermoids, and bilateral radial limb anomalies. Karyotype analysis revealed normal 46,XY with no evidence for Fanconi anemia. **Conclusion:** Tracheobronchial cartilaginous nodules are a novel finding expanding the clinical phenotype of OAVS. Morphogenesis of branchial arch and tracheoesophageal structures is dependent on proper interaction between mesenchymal and surrounding epithelial tissues during the fourth week of embryogenesis. We propose that this patient's anomalies illustrate a disruption of communication at the level of the cellular interface during this critical developmental stage. Tracheoesophageal anomalies and other malformations of mesenchymal derivatives may provide a clue to the etiology of this heterogenous disorder.

Program Nr: 808 from the 1999 ASHG Annual Meeting

Patent ductus arteriosus with a distinctive facial phenotype: Intrafamilial variability of the Char syndrome in a family with twins. *B.D. Friedman, L.A. Workman.* Perinatal and Pediatric Specialists Medical Group, Inc., Sacramento, CA.

We report on a family with patent ductus arteriosus and a distinctive facial phenotype in a mother and three of her four children. The findings in this family are similar to a rarely published syndrome first reported by Char (Birth Defects: Original Article Series XIV;6B:303-305, 1978), who described a family with patent ductus arteriosus and an unusual facial phenotype which included a short philtrum, duck-bill lips, ptosis, strabismus, and low set ears.

The affected individuals in this family include a 36 year-old mother, her 9 year-old son, and twin 5 year-old girls. All of the affected individuals required surgical repair of a patent ductus arteriosus in childhood; none was pre-term. In addition, a distinctive facial phenotype is present which includes a prominent forehead, upslanting palpebral fissures, a broad nasal bridge, a short, broad, flat nasal tip with anteverted nares, a short but prominent philtrum, and full lips with loss of the cupid's bow. The children have bifrontal narrowing with a prominent metopic area, and incurved fifth fingers. Short fifth fingers are present in one of the twins, her brother, and their mother. There is variable widening of the toes, which is most pronounced in the mother, and most commonly of the fifth toe. Other findings include a history of mild mixed hearing loss requiring hearing aids in the twins, who have severe receptive language, expressive language and articulation deficits. The mother had surgery for strabismus.

The findings in this family are consistent with previous reports of the Char syndrome, which includes autosomal dominantly inherited patent ductus arteriosus with a distinctive facial phenotype and variable other features, including hearing impairment, fifth finger clinodactyly, broad toes, and strabismus. Individuals presenting with a patent ductus arteriosus should be evaluated for similar phenotypic findings. Consideration should be given to audiologic and ophthalmologic testing in affected children and appropriate recurrence risk counseling should be offered.

Program Nr: 809 from the 1999 ASHG Annual Meeting

Metacarpophalangeal pattern profile (MCPP) analysis of Noonan syndrome. *D.D. Gale[1], G.A. Dahir[2], F.J. Meaney[3], R. Kumar[4], M.G. Butler[4].* 1) Col Allied Health & Nursing, Eastern Kentucky Univ, Richmond, KY; 2) University of Virginia, Charlottesville; 3) University of Arizona, Tucson; 4) The Children's Mercy Hospitals and Clinics, Kansas City, MO.

Metacarpophalangeal pattern profile (MCPP) analysis is an application of an anthropometric technique which provides a quantitative assessment of the amount and direction of abnormality in the hand skeleton. MCPP analysis was undertaken on 15 individuals (9 males; 6 females) with Noonan syndrome ranging in age from 0.1 to 36 years with a mean age of 11.6 years. This syndrome is associated with neck webbing, pectus excavatum, cryptorchidism, pulmonic stenosis, low posterior hairline, short stature and a particular facial appearance. The overall average Z score for the MCPP variables in individuals with Noonan syndrome was -2.1 and the range was -2.5 (metacarpal 2) to -1.5 (middle phalanx 5). The average pattern variability index, a measure of hand dysmorphogenesis, was 1.0. A value above 0.7 is considered abnormal. A Pearsonian correlation analysis was used to assess similarity between the mean pattern and each of the 19 individual patterns. Nine of 15 individuals with Noonan syndrome had significant positive correlations ($p < 0.05$) indicating homogeneity or similarity in hand patterns among Noonan syndrome subjects. A stepwise discriminant analysis was performed on 12 subjects with Noonan syndrome (8 males, 4 females; mean age = 7.3 years with age range of 0.1 to 13.5 years) and 41 controls (24 females, 17 males; mean age = 13.1 years with age range of 9.6 to 18 years). This analysis produced a discriminant function with age and one MCPP variable (middle phalanx 5) entered into the function. Although the hand pattern variability index indicated an abnormal MCPP, the multivariant analysis identified only one MCPP variable contributing to the overall difference between individuals with Noonan syndrome and the normative sample. The discriminant function will require testing with additional Noonan syndrome subjects for interpretation of its rate of correct classification of all individuals with Noonan syndrome.

Program Nr: 810 from the 1999 ASHG Annual Meeting

Three families with Perrault syndrome: Phenotypic variability versus genetic heterogeneity. *C.A. Gardiner[1], C.E. Chu[1], L. Al-Gazali[2], C.G. Woods[1], R.F. Mueller[1].* 1) Department Clinical Genetics, St James's University Hospital, Leeds, England; 2) Department of Paediatrics, United Arab Emirates University, United Arab Emirates.

The Perrault syndrome is an autosomal recessive condition. The diagnostic criteria are gonadal dysgenesis in the females and sensorineural hearing loss in both males and females. Other features reported include mental retardation and neurological abnormalities. It is unclear whether the Perrault syndrome is a single clinical entity. We report three families with female and male family members who are affected. The parents of the affected individuals are either first or second cousins. In the first family the affected female also has moderate mental retardation and a height greater than the 95th centile. Two of her brothers have sensorineural hearing loss but only one of the affected brothers has a height greater than the 95th centile and the probands mother has adult onset sensorineural hearing loss. In the second family the affected female has moderate mental retardation, as does her affected brother. In the third family, the affected female has a height below the third centile, but the height of her affected brothers is normal and all have normal intelligence. Twelve families have been previously reported in the literature. It has been suggested that the Perrault syndrome is either genetically heterogeneous or there is marked phenotypic variability but a third possible explanation of the features in the reported families is the occurrence of separate Perrault syndrome features occurring by chance in the same individual. In the families we report, chromosome analysis was normal and we did not find any mutations on sequencing Connexin 26 in affected family members. We conclude that the phenotypic spectrum of Perrault syndrome is not yet defined and it will await gene analysis and further family studies before the question of phenotypic variability versus genetic heterogeneity is answered.

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Program Nr: 811 from the 1999 ASHG Annual Meeting

Agenesis of the corpus callosum and spastic paraparesis. Observations in a mother and her two daughters. *R.J.M. Gardner[1], E. Storey[2], A.M. Walker[3], J. Heywood[4], K.J. Collins[5]*. 1) Victorian Clinical Genetics Services, Royal Children's Hospital, Melbourne; 2) Dept Neurosciences, Alfred Hospital, Melbourne; 3) Dept Paediatrics, Monash Medical Centre, Melbourne; 4) Dept Neurosciences, St Vincent's Hospital, Melbourne; 5) Dept Neurology, Royal Children's Hospital, Melbourne, Australia.

We have studied a family in which the mother and her two children have structural and/or functional white matter disease. The mother presented with clumsiness since childhood, proceeding to a spastic paraparesis in adult life, along with a bladder disturbance and an asymptomatic peripheral neuropathy. Her MRI scan was interpreted as normal. Both children have agenesis of the corpus callosum. The elder daughter had significant learning difficulties, and was held back for 1 year at school. At age 13, there were subtle signs to suggest an early spastic paraparesis. The younger daughter, had a diagnosis of hydrocephalus in infancy, and was shunted at 18 months. In spite of this, early milestones were reportedly normal, but subsequently her academic progress was poor. Gait and lower limb reflexes, at age 11, were normal. The family history is otherwise unremarkable. On the parsimonious assumption that the three phenotypes reflect the same genotype, we would propose that this may represent a previously undescribed neurogenetic condition, involving in particular a structural and functional white matter defect.

Program Nr: 812 from the 1999 ASHG Annual Meeting

Autosomal Dominant Gillespie syndrome in 4 generations with highly variable expressivity and incomplete penetrance. *J. Gaudelus, B. Heron, P. Bitoun.* Dept Pediatrie, NeuroPediatrie & Genet Med,CHU Paris-Nord, BONDY,93143cedex France.

Gillespie (1965) described 2 sibs with aniridia, cerebellar ataxia and mental retardation. A total of 21 patients have been described including 9 sporadic and 12 in 5 families; 3 including the princeps case have described affected sibs and 2 have described vertical maternal transmission. Etiology and transmission remains unclear and has generally been reported as autosomal recessive although no consanguinity has ever been reported. Most case reports mention aniridia but indeed description is that of iris hypoplasia since most patients have a minimal iris collarette. All patients have been reported with cerebellar ataxia and some with cerebellar hypoplasia , mental retardation and one with pulmonic stenosis. We report a case of iris hypoplasia in a 3 year old girl with hypotonia, psychomotor and speech delay and cerebellar ataxia with a normal M.R.I. and normal high resolution chromosomes all compatible with the diagnosis of Gillespie syndrome(GS). Family history is significant for a 30 year old paternal second cousin with identical irides without mental retardation or ataxia. A paternal great aunt with polycoria, and a paternal great grandmother with a narrow anterior chamber. A paternal first cousin has mental retardation but his eye examination is not known and his status is unclear. This family thus shows a dominant transmission of GS with at least 4 affected members in 4 generations all having isolated iris anomalies including polycoria, narrow anterior chamber except the proband who is the only affected with both iris and cerebellar and mental symptoms. It seems that there are 2 unaffected transmitting males who are obligate carriers and incomplete penetrance in the affected. All reported familial cases are compatible with dominant transmission, the affected sibships could possibly be due to either minimal or non penetrance in one parent or germline mosaicism. Glaser et al 1994 have searched for mutation in the PAX6 gene in 3 families with GS with negative results while Dollfus et al (1998) have reported a patient with GS and a t(X;11)(p22.32;P12) de novo translocation without detectable anomaly in the PAX6 coding sequence.

Program Nr: 813 from the 1999 ASHG Annual Meeting

Unusual distribution of acanthosis nigricans in the Crouzonodermoskeletal syndrome. *J.T. Goodrich[1], S. Orlow[2], D. Staffenberg[1], R. Marion[1], S. Eisig[1], A. Shanske[1].* 1) Depts. of Neurological Surgery, Pediatics, Plastic Surgery, and Dentistry, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY; 2) Dept. of Dermatology, NYU Medical Center, New York, NY.

Acanthosis nigricans (AN) consists of thickening and hyperpigmentation of the skin usually confined to the flexural areas of the body and rarely the entire body. The oral mucosa is affected in 25% of cases and involvement of the esophagus has been reported. It may occur as part of the Crouzondermaoskeletal syndrome due to a mutation in FGFR3, ala391glu. In this disorder, the Crouzonoid phenotype is combined with AN, jaw cementomas, vertebral alterations and hydrocephalus. We have observed AN in the meninges of a girl with Crouzon syndrome (CS).

KS is an 11 year old who has already undergone a number of procedures because of her Crouzon syndrome including a tracheostomy and VP shunt placement. Her physical examination prior to her recent surgery revealed a stigmatized youngster whose height was 132 cm(10%), weight 40 kg(50-75%), and HC was 51.5 cm(25%). She had turricephaly with deep depressions above the supraorbital ridges, hypertelorism, a downward obliqity of the palpebral fissures and severe midfacial hypolplasia. The intraoral examination revealed an underbite, byzantine arch and bifid uvula. AN involved the chin, the commisures of the mouth, median canthi, upper lids, neck, chest and axillary areas. She also had a number of macular nevi. She underwent a LeForte III with distraction in conjunction with a calvarial bone graft and bilateral canthoplasties. At surgery, striking thickening of the dura and difficulty elevating the forehead flap was noted. The tissue around the eyes was friable and thin. A number of pigmented areas of the meninges were visible through the craniotomy holes.

AN is associated with several congenital disorders including CS. The AN associated with CS is atypical in several ways. Histochemical analyses using in situ hybridization has shown that the FGFR3 gene is expressed in many areas of the brain. Our observation confirms it is also expressed in the meninges.

Program Nr: 814 from the 1999 ASHG Annual Meeting

Two cases of partial trisomy 8 and 14 (8q24.1®qter and 14pter®q22) with semilobar holoprosencephaly and comparisons to Seckel syndrome. *G.C. Gowans[1,3], S. Goncalves[5], A.M.V. Duncan[2,3], P. Eydoux[4], A.S. Teebi[5]*. 1) F. Clarke Fraser Clinical Genetics Unit, Division of Medical Genetics, Montreal Children's Hospital, McGill University, Montreal, QC, Canada; 2) Department of Pathology, Montreal Children's Hospital, McGill University, Montreal, QC, Canada; 3) Department of Human Genetics, McGill University, Montreal, QC, Canada; 4) Biologie du Developpement, Hopital Roberte-Debre, Paris, France; 5) Department of Genetics, The Hospital for Sick Children, Toronto, ON, Canada.

We report sibs with +der(14)t(8;14)(q24.1;q22)mat . The anomaly arose from a 3:1 meiotic segregation of a maternal balanced reciprocal translocation. The patient's sister, mother and maternal grandmother are known to be balanced translocation carriers. Their karyotypes are 46, XX, t(8;14)(q24.1;q22). The first unbalanced case was detected through amniocentesis with a karyotype of 47, XY, +der(14)t(8;14)(q24.1;q22)mat. This male neonate had many congenital abnormalities including microcephaly, low set ears, large beaked nose, micrognathia, cleft palate, cryptorchidism, camplodactyly, bilateral club feet and semilobar holoprosencephaly on MRI. He died at 27 days of respiratory failure. The second case, a female, also has tertiary trisomy 8 and 14 and has a very similar phenotype with microcephaly, lowset ears, a beaked nose with a wide malar region, retrognathia with cleft hard palate, camplodactyly, bilateral club feet and semilobar holoprosencephaly. Comparisons are made with other cases of partial duplications of 8 and 14 and the relation of these cases to Seckel and Seckel-like syndromes is discussed. The phenotypes of these infants have much in common with the autosomal recessive Seckel type dwarfism that is characterized by severe short stature, microcephaly, prominent beaked nose, and receding lower jaw. We also suggest that holoprosencephaly could be an additional feature of trisomy 8q24®ter and/or 14pter®q22.

Program Nr: 815 from the 1999 ASHG Annual Meeting

AN AUSTRALIAN CADASIL PATIENT WITH A NOVEL NOTCH3 MUTATION. *R.F Grigg[1], A.A. Sullivan[2], R.P. Curtain[1], L.R. Griffiths[1]*. 1) Genomics Rsc Ctr, Sch Hlth Sci, Griffith University-Gold Coast, Southport QLD, Australia; 2) Department of Medicine, Royal Brisbane Hospital, Herston, Qld.

CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) is a dominantly inherited disease of the small vessels of the brain. Recent studies by Joutel et al1 have indicated that mutations within the Notch3 gene are involved in families with this disorder. Here we describe the clinical features of a patient from a previously reported Australian CADASIL kindred with a novel mutation of the Notch3 gene. Using PCR techniques to amplify the Notch3 gene, we performed direct mutation analysis via sequencing. A base substitution mutation involving a G to T transition was identified. The mutation results in an amino acid change from a Cysteine to a Phenylalanine and is found in exon 4. A recent study by Joutel et al 2 found a clustering of mutations in this region, all resulting in the loss or gain of a cysteine residue. These typesof mutation, within the EGF-like domain, are thought to be involved in aberrant intramolecular disulphide bonds, resulting in abnormal folding of Notch3 or inappropriate bond formation with other cysteine-containing proteins.DNA from a sibling of this patient, who has also been diagnosed with CADASIL is also currently being used for mutation analysis. Other members of the family are undergoing genetic counselling before considering testing. (1) Joutel et al 1997 Ann N Y Sci826:213-217 (2) Joutel et al 1997 Lancet350:1511-1515.

Program Nr: 816 from the 1999 ASHG Annual Meeting

Neurodevelopmental Assessment and Functioning in Five Young Children with Smith-Magenis syndrome (SMS).

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Smith-Magenis syndrome (SMS), a probable contiguous gene syndrome due to del 17p11.2, is associated with a distinctive phenotype of characteristic physical features, developmental and neurobehavioral abnormalities, and speech & language delay. Limited studies have been reported on young children. We developed a comprehensive test battery to assess the early cognitive and behavioral phenotype of SMS. A neurologic, developmental, language, and oral motor assessment was administered to five children with SMS ages 21- 28 months. All children had neurologic abnormalities including hypotonia, motor delays, at least one feature of peripheral neuropathy (decreased DTRs, pes planus/cavus, decreased pain sensitivity, 6-8 Hz upper extremity tremor, distal muscle weakness), and sleep disturbance. Psychometric testing revealed cognitive functioning in the moderately delayed to low average range. Expressive verbal language was more delayed than receptive language in all children. All exhibited limited vocalizations and poor vocal sound production but had begun to use simple signs and gestural communication. Oral motor findings were consistently found: all had open mouth posturing, exaggerated lingual papilla, anterior tongue carriage, & decreased lingual movements; 3/5 had downward slanting cupid bow mouth and palatal abnormalities. Orientation/engagement and emotional regulation scores were within normal limits in all cases, yet mild-moderate autistic-like behaviors also were seen in four. Videotaped samples will be shown to illustrate the salient features of SMS behavior. Due to decreased verbal skills, maladaptive behavior, and developmental delays, a comprehensive battery is useful to assess the range of cognitive and neurobehavioral abnormalities in SMS to facilitate early identification and appropriate educational, behavioral, and speech interventions.

Program Nr: 817 from the 1999 ASHG Annual Meeting

47,XXX with Mullerian Agenesis. *C. Guze, R. Hassan, A. Adeniji, F. Hodges, D. Estrada-Smith.* Obstetrics and Gynecology, King Drew Medical Center, Los Angeles, CA.

We report on a 27-year-old, white Hispanic female referred from the fertility clinic because of primary amenorrhea. Physical examination revealed a blind 3 cm vaginal pouch with no cervix, Tanner stage 3 breasts, and scant pubic and axillary hair. She has a flat facies, short fingers and toes, and her toes are widely separated. Our patient weighs 207 lbs and is 5 ft 5 in which is several inches taller than her 3 sisters. Her IVP showed normal urinary anatomy. An MRI of the abdomen and pelvis showed an absence of the uterus and hypoplasia of the vagina. The ovaries are present bilaterally. The left ovary, 2.5 cm x 2.0 cm, is located anteriorly in the pelvis and the right, 4.5 cm x 4.9 cm, is posteriorly located. Both contained normal appearing follicles. The patient experiences monthly pelvic pain. The MRI showed no evidence of any abnormality of the liver, spleen, kidneys, pancreas or adrenal glands. TSH was normal (1.77 IU); FSH (3.3 mIU/ml) and LH (8.2 mIU/ml) were normal for follicular and luteal phases. PRL (21.7 ng/ml), E2 (112 pg/ml) and T (31 ng/dl) levels were within normal limits. In both blood and fibroblast chromosome studies, a 47,XXX karyotype was observed.

The patient stopped going to school when she was 16 years old and still in the 6th grade. She reports that she has a speech problem and difficulty expressing herself. She describes herself as shy and often depressed. She feels she is different from her siblings in social skills and ability to retain information. She expressed relief at finding there was an explanation for her differences.

Our patient manifests several clinical features often associated with the 47,XXX condition. These include learning and language problems, depression, shyness, increased height relative to female sibs, and fertility problems. However, her infertility is unique because it is due solely to mullerian agenesis without ovarian dysgenesis. She has complete absence of the fallopian tubes, uterus, and the internal portion of the vagina along with functional ovaries. Unlike other triple-X patients reported to have mullerian anomalies, there is no evidence of urinary tract malformation.

Program Nr: 818 from the 1999 ASHG Annual Meeting

Prenatal diagnosis of trisomy 8 mosaicism. *J. Habecker-Green, R. Naeem, S. Pflueger, J.P. O'Grady, G. Markenson, G.M. Cohn.* Baystate Medical Ctr, Springfield, MA 01199.

Trisomy 8 mosaicism is a variable condition that may include multiple anomalies and mental retardation. Prenatal diagnosis for this condition has rarely been reported. We present two cases of prenatal diagnosis for trisomy 8 mosaicism.

Case 1: A 16-year-old Puerto Rican female presented for routine prenatal ultrasound. The fetus had Dandy-Walker malformation, choroid plexus cysts, absence of the inferior vermis, hydrocephalus, mild pyelectasis, and possibly a small abdominal wall defect. Amniocentesis was performed, but cellular culture failed. Repeat amniocentesis or PUBS was declined. At 36 weeks gestation, a 2.2 kg female infant was delivered. She had cleft palate and microcephaly with brain anomalies including Dandy-Walker malformation, hydrocephalus, schizencephaly, lissencephaly, and intraparenchymal calcifications. Renal ultrasounds and spine films were normal Peripheral blood karyotyping was significant for 47,XX,+8[2]/46,XX[121] mosaicism confirmed by FISH. Cerebrospinal fluid cultures for karyotypic analysis were attempted and were unsuccessful. Additional karyotypic studies were declined.

Case 2: A 29-year-old female of northern European ethnicity presented for routine prenatal ultrasound. The fetus was noted to have unilateral ventriculomegaly, unilateral hydronephrosis, a disorganized spinal colums (possibly secondary to a hemivertebra), and single umbilical artery. Amniocentesis was performed and was significant for 47,XY,+8[5]/46,XY[20] mosaicism. The parents were counseled about the variable nature of trisomy 8 mosaicism and chose to end the pregnancy. Post mortem evaluation was notable for a wide head and dysmorphic face, low set ears, large nuchal fold, micrognathia, abnormal rotation of the heart, a 9th thoracic hemivertebra, and deep palmar and plantar creases. Trisomy 8 was seen in all studied thymus cells, in 70% of skin cells, in 20% of villi, and was not seen in brain cells.

Program Nr: 819 from the 1999 ASHG Annual Meeting

New x-linked MR/MCA syndrome associated with cleft lip/palate, upslanted/short palpebral fissures, high nasal bridge, prominent nasal tip, inguinal hernia and minor digital defects. *B.D. Hall, J.M. Robl.* Dept Pediatrics/Genetics, Univ Kentucky Medical Ctr, Lexington, KY.

There are approximately 178 recognized disorders with x-linked mental retardation (Am J Med Genet 83:237, 99). Seventy-five (42%) of these are associated with multiple congenital anomalies (MR/MCA) which allows for more rapid, specific syndrome identification. Only 9% of these x-linked MR/MCA syndromes are associated with cleft lip and/or cleft palate. We report a new x-linked MR/MCA syndrome in three males of a single family. Two of the males, ages 10 years and 16 months, are brothers and the third male is a maternal first cousin through normal female carriers. All three affected males share moderately severe mental retardation, normal prenatal growth parameters, upslanted eyes, short palpebral fissures, prominent nasal tip, and marked delay in speech. Two of the three males have clefts (cleft lip in one/cleft palate in the other), increased inner canthal distance, high-wide nasal bridge, short 5th fingers, low set ears, and inguinal hernias. The following features are found in one of each of the males: postnatal growth deficiency, left helical pit, kyphosis, cubitus valgus, small thumbs, left club foot, multicystic left kidney, and undescended testes. Chromosomes were normal and a brain MRI in one male revealed asymmetric white matter. We could not find a similar phenotype in any of the reported x-linked MR/MCA syndromes. These three males have a distinct recognizable facial gestalt associated with oral clefts. Further studies to include gene localization are planned.

Program Nr: 820 from the 1999 ASHG Annual Meeting

FGFR2 mutations in patients with Pfeiffer syndrome type III. *K. Hedrich, H. Thiele, A. Rühl, I. Hansmann, U. Hehr*. Humangenetik, Martin-Luther-Universität, Halle, Germany.

Craniosynostosis, the premature fusion of one or several cranial sutures, is with a prevalence of appr. 1 in 3,000 one of the most common craniofacial anomalies at birth. Recently, mutations in three of the four human fibroblast growth factor receptor genes (FGFRs) have been shown to account for appr. 15-20% of all cases with craniosynostosis. Here we report the results of a screening for FGFR mutations in 5 familial cases and 10 sporadic patients with craniosynostosis, referred to our department for genetic counseling. Two of the patients were identified as Pfeiffer syndrome Type III. In addition to bilateral coronal craniosynostosis with short anterior cranial base and ocular proptosis, both patients presented with developmental delay, hydrocephalus internus, hearing deficit and short stature. In both patients previously reported mutations affecting exon IgIIIc of FGFR2 were identified. The first patient died at the age of 34 months following repeated craniosurgery, the clinical course was complicated by dyspnoe resulting from choanal stenosis and stenosis of the bronchi. She was found to carry the common FGFR2 mutation Cys342Arg within exon IgIIIc, which had previously been reported in more than 20 patients with CS, PS or JWS. The second patient presented with localized tracheostenosis and multiple orthopedic problems including hip dysplasia, severe scoliosis and limited extension of the elbows. Mutation analysis confirmed an A(-2)G substitution at the acceptor splice site of exon IgIIIc. Comparison of the clinical features of both patients with the previously reported cases further underlines the wide phenotypic spectrum of identical FGFR2 nucleotide substitutions, most notably in exon IgIIIc, and supports the idea of additional genetic and/or environmental factors modifying the extend of the initial mutation effect.

Program Nr: 821 from the 1999 ASHG Annual Meeting

Silver-Russell syndrome and cystic fibrosis caused by maternal uniparental disomy 7. *U. Hehr[1], S. Brömme[2], S. Dörr[1], M. Hagemann[1], U. Preiss[2], I. Hansmann[1].* 1) Humangenetik, Martin-Luther-Universität, Halle, Germany; 2) Kinderklinik, Martin-Luther-Universität, Halle, Germany.

Maternal uniparental disomy 7 (mUPD7) has been reported so far in two patients with cystic fibrosis (CF; Spence et al., 1988, Voss et al., 1989). Prenatal and postnatal growth retardation in both patients were more severe than expected in patients with CF alone. This marked growth retardation, typically combined with asymmetry and a distinct small triangular face, is recognized today as a significant feature of Silver-Russell syndrome (SRS). About 10% of SRS patients are identified to result from mUPD7, which prompted the search for differentially imprinted growth related genes on chromosome 7. Here we describe a third patient with mUPD 7 causing the combination of SRS and CF. He presented with intrauterine growth retardation, postnatal failure to thrive and distinct craniofacial features. No asymmetry was noted. Two weeks post partum an exocrine pancreas insufficiency was diagnosed. Weight gain resumed under pancreas enzyme substitution, although the patient at the age of 2 years still remains under the third percentile for both weight and height. Mutational screening of the CFTR gene revealed homozygosity of the patient for the common CFTR mutation DF508, while only his mother was found to be heterozygous for DF508. Microsatellite analysis confirmed maternal uniparental isodisomy 7 and was consistent with paternity for other chromosomes. With this report we want to emphasize the importance of screening for UPD 7 in patients with CF and severe growth retardation. Additional features of SRS might not be present at the time of examination. Finally, the diagnosis of UPD 7 in patients with CF confirms a novel epigenetic mechanism for the inheritance of CF, which provides the affected parents with a much better perspective for subsequent pregnancies when compared to the common recurrence risk for CF of 25%.

Program Nr: 822 from the 1999 ASHG Annual Meeting

A second family linked to spinocerebellar ataxia 5. *A. Herman[1], G. Stevanin[1], G. Cancel[1], O. Didierjean[1], A-S. Lebre[1], Y. Agid[1,2], A. Brice[1,2], A. Durr[1,2]*. 1) INSERM U289, Hopital de la Salpetriere, Paris, France; 2) Federation de Neurologie, Hopital de la Salpetriere, Paris, France.

Spinocerebellar ataxia type 5 (SCA5), one of the genetically heterogeneous autosomal dominant cerebellar ataxias, was assigned to chromosome 11 in a single family descending from the grand parents of President Lincoln. We report a second, apparently unrelated, SCA5 family, of French origin. Although the clinical pictures of the two families are similar, we show that the slowly progressive cerebellar ataxia that characterizes the phenotype in SCA5 patients can be associated with other signs such as nystagmus, myokimia and decreased vibration sense, thus indicating that the clinical picture of these patients can overlap with those carrying SCA1-7 mutations. Furthermore, the age at onset (mean of 27 years), that varies from the second to the fourth decade, is discordant with the late onset that was thought to characterize pure cerebellar ataxias. Magnetic resonance imaging showed a marked global cerebellar atrophy, even after a short disease duration, similar to SCA6. Finally, we did not find evidence of maternal anticipation, as previously described (Ranum et al, 1994), and no CAG repeat expansions or polyglutamine-containing-protein were detected in patients using RED or 1C2-western-blot techniques.

Program Nr: 823 from the 1999 ASHG Annual Meeting

Resolution of hematologic abnormalities not due to somatic mosaicism in a male with Fanconi anemia. *J.H. Hersh[1], A.D. Auerbach[2]*. 1) Dept Pediatrics/CEC, Univ Louisville, Louisville, KY; 2) Laboratory of Human Genetics & Hematology, The Rockefeller University, New York, N.Y.

Fanconi Anemia (FA) is an autosomal recessive disorder characterized by various congenital malformations and a predisposition to bone marrow failure and leukemia. At least 8 complementation groups have been identified, and both clinical and genetic heterogeneity exist in affected individuals. Hypersensitivity of FA cells to the clastogenic effect of diepoxybutane (DEB) serves as a cellular marker for the disorder. In a portion of FA patients, hematologic mosaicism may lead to normal hematopoiesis and improvement in hematologic status. We present a male with FA who was suspected of being affected at 8 years of age based on the presence of hypoplastic thumbs, microcephaly, short stature, cryptorchidism, hyperpigmentation, mild intellectual deficits, vesicoureteral reflux and evidence of bone marrow suppression. At that time, WBC was 3100, Hgb 10 & platelet count 42,000. At 19 years, his hematologic status had normalized with a WBC 7600, Hgb14.3 and platelet count 172,000. Exposure of peripheral blood lymphocytes to DEB at 8 and 19 years of age resulted in 7.6 and 8.5 mean breaks/cell, and 92% and 100% aberrant cells respectively. A variant in *FANCG* was discovered in this patient (IV12 -38 -(-28)del); the pathogenicity of this variant is not determined as yet. Normalization of hematologic findings in an FA patient without somatic mosaicism has yet to be described. Additional complementation studies and mutation analysis are reviewed in order to elucidate the mechanism for natural gene therapy in this FA patient.

Program Nr: 824 from the 1999 ASHG Annual Meeting

Dysmorphic features, multiple congenital anomalies, and preaxial polydactyly of feet: Diabetic embryopathy or a malformation syndrome? *T. Heshka[1,2], K. Chorneyko[1,2], B. Brennan[1,2], M.J.M. Nowaczyk[1,2]*. 1) Hamilton Health Sciences Corporation; 2) Faculty of Health Sciences, McMaster University, Hamilton, ON, Canada.

This male infant was born at 28 weeks gestation to a 23 y.o G1 with poorly controlled IDDM. Severe oligohydramnios and fetal anomalies were noted antenatally. The baby had micrognathia, low set ears, high, broad and flat forehead, flat-appearing face, hypoplastic nose with anteverted nares. There was a cleft of the lower alveolar ridge, a bifid uvula, and a normal tongue. There was severe bowing of the legs, severe equinovarus deformities of both ankles and metatarsus adductus of both feet. There was pre-axial polydactyly of both feet with proximally placed great toes. The hands and upper limbs were normal. External genitalia were male with hypospadias and undescended testes. Radiographs showed 11 pairs of ribs, two hemivertebra with associated bifid ribs, and bilateral parietal foramina. There was a single bowed bone in the left lower leg and two bowed bones in the right lower leg. Autopsy showed hypoplasia of the pulmonary valve and pulmonic trunk, right aortic arch with mirror image branching, an aorto-pulmonary window with incomplete truncus arteriosus, and a type C T-E fistula. Kidneys and ureters were absent and bladder was hypoplastic. Pancreas showed islet cell hyperplasia. Neuropathologic examination showed a 3.0 x 4.0 mm hamartoma in the septum pellucidum. There was placentomegaly and focal amnion nodosum. Karyotype was normal male; DNA analysis for SOX9 mutations was negative.

The features presented here are suggestive of VACTERL association, however, the facial dysmorphism and polydactyly is against this diagnosis. Preaxial toe polydactyly is a recognized feature of diabetic embryopathy as are renal agenesis and vertebral and cardiac malformations. However, this infant had a number of other malformations and dysmorphic features that suggested an underlying syndrome. The differential diagnosis includes acrocallosal syndrome, oral-facial-digital spectrum, camptomelic syndrome, Pallister-Hall syndrome, diabetic embryopathy, and a newly recognized syndrome.

Program Nr: 825 from the 1999 ASHG Annual Meeting

Familial Charcot-Marie-Tooth with spasticity and additional features. *F.M. Hisama, D.S. Russell, E. Auld, J.M. Goldstein.* Dept of Neurology, Yale Univ Medical School, New Haven, CT.

Charcot-Marie-Tooth (CMT) is a genetically heterogenous group of peripheral nerve disorders characterized by pes cavus, hammer toes, and distal atrophy. CMT is usually inherited an an autosomal trait. Mutations in connexin-32 cause an X-linked dominant form of CMT, with milder clinical and electrophysiological features in females compared with their male relatives. We present a family with 5 affected individuals (age 10-55 years, 3 females and 2 males) in 3 generations. Each has clinical features of CMT, however, both clinically and by nerve conduction velocity, males are more severely affected than females. CMT is rarely associated with upper motor neuron signs, yet the 4 subjects examined have significant spasticity and hyperreflexia. Additional features in more than one family member include dysarthria and strabismus. The most severely affected male has mental retardation, low set ears,severe scoliosis, and multiple contractures. Brain MRI of the proband showed mild, diffuse increased white matter signal with normal corpus callosum. Evoked potentials showed combined peripheral and central neuropathy. Electromyography/nerve conduction studies of multiple family members showed demyelinating and axonal sensorimotor neuropathy, more severe in the males. DNA testing for PMP-22 duplication in CMT-1A and mutations in connexin-32 was negative. Very long chain fatty acids and phytanic acid were normal. Because of overlap with spinocerebellar ataxias, DNA testing for SCA1,2 and 3 was performed and was negative. This family differs from others with hereditary motor and sensory neuropathies by the presence of spasticity, hyperreflexia, and additional features. The inheritance pattern is suggestive of X-linked dominant inheritance or autosomal dominant inheritance with sex-influenced expression.

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Program Nr: 826 from the 1999 ASHG Annual Meeting

Robin sequence with distichiasis: a new dominant syndrome. *R.J. Hopkin[1,2], C.E. West[3]*. 1) Div Human Genetics, Children's Hosp Medical Ctr, Cincinnati, OH; 2) Craniofacial Center, Children's Hosp Medical Ctr, Cincinnati, OH; 3) Div Ophthamology, Children's Hosp Medical Ctr, Cincinnati, OH.

The triad of micrognathia, glossoptosis and cleft palate define Robin sequence. Distichiasis, two rows of eyelashes, is a rare congenital malformation that has been reported as an isolated malformation, in association with congenital lymphedema, and as a part of several rare genetic syndromes. We have recently evaluated a family with Robin sequence and distichiasis inherited as a dominant condition. This combination has been reported in only one previous patient. **Case report:** The proband is a 2 year old female with micrognathia, cleft palate, and distinct facial features including a flat profile, down slanting palpebral fissures, and epicanthic folds. Growth and development have been normal. An ophthalmologic evaluation revealed bilateral distichiasis with no evidence of myopia. **Family history:** The patient's father was born with micrognathia, cleft palate, distichiasis, and sensory neural hearing loss (SNHL). The paternal grandmother has micrognathia, cleft palate, and distichiasis. A paternal uncle has micrognathia, cleft palate and SNHL. He is not known to have distichiasis. The paternal grand father has SNHL, but is otherwise without congenital malformations. There is no history of lymphedema or high myopia in any family member. **Discussion:** We report on a family with a previously undescribed syndrome characterized by Robin sequence, distichiasis, and distinct facial features that are inherited as a dominant trait. Important negatives include the absence of lymphedema, high myopia, or other major malformations. There is a great deal of overlap with non-occular Stickler syndrome, however, distichiasis is not seen in that condition. It is possible that some individuals previously diagnosed with non-occular Stickler syndrome had the condition reported in this family. It is also interesting to note that, with the exception of isolated distichiasis, all of the syndromes associated with distichiasis have cleft palate as an occasional feature. This syndrome may reveal insights into the genetic and developmental factors that lead to cleft palate and facial dysmorphogenesis.

Program Nr: 827 from the 1999 ASHG Annual Meeting

Trisomy 21 and Ebstein Anomaly. *M.J. Huggins, J. Mernagh, J. Xu, M.J.M. Nowaczyk.* Hamilton Health Sci Corp, Hamilton, ON, Canada.

Ebstein anomaly (EA) consists of downward displacement of an abnormal tricuspid valve into the right ventricle with resultant enlargement of the right atrium and tricuspid valve incompetence. Although there are reports of EA following fetal exposure to lithium, EA malformation is sporadic and rare. Approximately 40 per cent of fetuses and children with Down syndrome (DS) have a cardiac anomaly; there are two cases of EA and DS reported in the literature. We report a fetus with trisomy 21 who had EA diagnosed during a prenatal sonographic evaluation at 25 weeks of gestation. This finding was confirmed by fetal echocardiography.

A 42 year old G1 was referred for a genetic amniocentesis because of advanced maternal age and triple screen positive for DS. The fetal karyotype was 47,XY,+21. Sonographic examination at 25 weeks of gestation showed thickening of the nuchal fold measuring 10.5 mm (normal <6 mm), and cerebral ventriculomegaly [posterior horns of the cerebral ventricles measured 11 mm in maximum diameter (normal<10 mm)]. The fetal heart circumference was 106 mm corresponding to an estimated gestation of 29 weeks. The right atrium was enlarged with an apically displaced tricuspid valve. The interventricular septum was intact. Fetal echocardiogram confirmed the finding of EA. Bilateral pleural effusion was also noted. The pregnancy ended at 33 weeks of gestation following the ultrasound finding of intrauterine fetal demise. Autopsy was not performed.

To our knowledge, EA has previously been reported in association with DS in two patients; in both cases EA was an incidental finding and had no clinical sequelae. These three cases suggest that DS should be considered in the differential diagnosis of EA detected antenatally.

Program Nr: 828 from the 1999 ASHG Annual Meeting

Skewed X-chromosome inactivation in 47,XXY and 48,XXYY patients: potential explanation for phenotypic variation among Klinefelter individuals. *Y. Iitsuka[1, 2], A. Bock[1], D.D. Nguyen[1], J.L. Simpson[1], F.Z. Bischoff[1]*. 1) OB/GYN, Baylor College of Medicine, Houston, TX; 2) OB/GYN, Chiba University School of Medicine, Chiba, Japan.

Klinefelter syndrome occurs in approximately 1:800 male births, and accounts for about 10-20% of males attending infertility clinics. Most studies have shown no obvious phenotypic differences between Klinefelter syndrome patients in which the extra X-chromosome is of paternal or maternal origin, but a minority of 47,XXY patients are adversely affected clinically and intellectually. The explanation for this phenotypic variation is not apparent. We hypothesize random or skewed X-inactivation in correlation to parental origin of the X-chromosomes. EXPERIMENTAL RATIONALE- We determined the parental origin and inactivation status of the X-chromosomes in 16 cytogenetically confirmed 47,XXY cases, two 48,XXYY cases and one 46,XY/47,XXY case. Parental origin of X-chromosomes was determined using fluorescent-based PCR for nine highly polymorphic markers specific to the X-chromosome. The human androgen-receptor methylation assay was then used to measure quantitatively X-inactivation status in subjects and their parents (controls). RESULTS- In 10 (53%) of 19 cases, both X-chromosomes were of maternal origin; in the remaining 9 (47%), one X was of maternal and one was of paternal origin. Of the 19 cases, 15 were informative at the androgen-receptor locus; in 4 (27%) of the 15, nonrandom X-inactivation was observed as defined by greater than 80% preferential inactivation involving one of the two X-chromosomes. The two 48,XXYY cases each showed one X of maternal origin (X^m) and one as paternal origin (X^p); in both cases preferential paternal X-chromosome inactivation existed. Two 47,X^mX^mY also showed preferential inactivation in one of the two X's, based on polymorphic marker analysis. CONCLUSION- Our results provide novel evidence for skewed X-inactivation in Klinefelter syndrome. This mechanism could explain the wide range of mental deficiency and phenotypic abnormalities observed in the disorder. Further studies correlating clinical features with parental origin and X inactivation status are in progress.

Program Nr: 829 from the 1999 ASHG Annual Meeting

Overlapping features of Prader-Willi syndrome and trisomy 18 during infancy. *H.A. Ishmael[1], L.M. Pasztor[1], P.G. Rothberg[1], J. Pfotenhauer[2], V. Hannig[2], M. Summar[2], M.G. Butler[1].* 1) Section of Medical Genetics and Molecular Medicine, The Children's Mercy Hospitals and Clinics, Kansas City, MO; 2) Division of Medical Genetics, Department of Pediatrics, Vanderbilt University, Nashville, TN.

Prader-Willi syndrome (PWS) and trisomy 18 share a number of features in the neonatal period. We recently evaluated three hypotonic infants with dysmorphic features who demonstrate common characteristics. The first, a 20-month-old female, who was born at term, was referred due to developmental delay and growth retardation. Decreased fetal movement and a choroid plexus cyst was identified by routine prenatal ultrasound. She was hypotonic with significant neonatal feeding difficulties. She had a narrow bifrontal diameter, dolicocephaly with mild ridging of the sagittal and metopic sutures, almond shaped eyes, a small mouth with a thin upper lip, a short nose, narrow hands, a small VSD, hypotonia, and developmental delay. Chromosome testing with FISH analysis was performed because of the clinical impression of PWS. Surprisingly, trisomy 18 mosaicism [47,XX, +18(23 cells)/46,XX(27 cells)] was found with no evidence of a 15q11-13 deletion. DNA methylation analysis with the SNRPN probe was normal. The second patient was thought initially to have trisomy 18 during the newborn period. He was severely hypotonic and presented at birth with a narrow bifrontal diameter, a small mouth, a prominent occiput, dorsiflexion of great toes, low set ears, bilateral cryptorchidism, and a high arched palate. He was found to have a deletion of 15q11-13, which was confirmed by FISH. A third hyptonic newborn with maternal disomy 15 was also initially thought to have trisomy 18. This female was hypotonic and had a narrow bifrontal diameter, a down turned mouth, almond shaped eyes, narrow hands, and a weak cry. These subjects illustrate the clinical similarities between PWS and trisomy 18 during infancy. One must proceed cautiously with the initial counseling and management of these infants. The prognosis and etiology behind these two conditions are markedly different.

Program Nr: 830 from the 1999 ASHG Annual Meeting

Phenotypic features of a patient with mosaic trisomy 18 and 45,X; comparison with three previous cases. *K.E. Jackson[1], M. Prieto-Carrasquero[1], S. Li[1,2], M. Marble[1]*. 1) Human Genetics Program, Tulane Univ Medical Sch, New Orleans, LA; 2) Department of Medical Genetics, University of South Alabama, Mobile, AL.

We report a 15-month-old female who was initially referred to genetics at six months of age because of developmental delay, and slightly abnormal urine organic acids at an outside laboratory. Growth parameters were in the 25th-50th percentile. She had micrognathia, narrow palate, downslanting palpebral fissures, cutis marmorata, hypotonia, and mild developmental delay. We analyzed urine organic acids, plasma amino acids, biotinidase enzyme analysis, and ammonia, which were non-diagnostic. The developmental delay persisted, and at 14 months, a karyotype surprisingly revealed 47,XX,+18, in 50 out of 50 cells analyzed. Echocardiogram, renal and abdominal ultrasounds were all normal. Since her phenotype was not indicative of trisomy 18, we repeated the karyotype in peripheral blood and skin fibroblasts. A repeat peripheral blood karyotype showed mosaicism for trisomy 18 and 45,X. Of 121 cells analyzed, 100 cells had trisomy 18, while 21 cells had 45,X. Interphase and metaphase fluorescent *in-situ* hybridization (FISH) are being performed for further analysis. The FISH results and the fibroblast cytogenetic analysis result will be presented.

In conclusion, we report a patient with a rare chromosomal abnormality. There are three previous case reports with mosaic 45,X and trisomy 18 in distinct cell lines. However, these individuals had a phenotype similar to Turner syndrome, while our patient has developmental delay, without stigmata of Turner syndrome. The percentage of cells with 45,X was higher in these reports than in our patient.

Program Nr: 831 from the 1999 ASHG Annual Meeting

A novel method for quantifying facial asymmetry. *P.L. Jamison[1], R.E. Ward[2], E.T. Everett[2].* 1) Anthropology, Indiana University, Bloomington, IN; 2) Oral Facial Genetics, Indiana School of Dentistry, Indianapolis, IN.

Assessment of facial asymmetry may be useful in exploring dysmorphogenesis. We hypothesize that disruptions in developmental stability may be evidenced by abnormal degrees of facial (fluctuating)asymmetry. Few studies have used direct measurements of the face to assess asymmetry because normal ranges of variation in facial asymmetry were unknown. In this study we describe two measures of overall asymmetry: a measure of total asymmetry (TA) based on summed differences for fourteen paired craniofacial anthropometric measurements and a weighted summed asymmetry, (WA) based on the absolute (nonsigned) differences but adjusted for the size of the measure. Using a large (N = 1312) reference data set of normal individuals, we calculated asymmetry values (TA and WA) for every individual in this sample. For TA the resultant distribution was non-skewed (S=.075; SE=.068) but was kurtotic (K=1.247; SE=.135) while the distribution for WA was both skewed (S=.942; SE=.068) and kurtotic (K=2.300; SE=.135). Following accepted statistical conventions for such nonnormal distributions, we utilized the 3rd and 97th percentiles of these distributions to define normal ranges of variation for each value. We also calculated TA and WA for 24 individuals with recognized syndromes manifesting various degrees of craniofacial dysmorphology and compared the results to our reference base asymmetry norms. Nineteen (79.2%) of the syndromic individuals fell outside the normal range on one or both of the asymmetry measures. Furthermore the ability to detect abnormal degrees of facial asymmetry may be useful in testing the theory that conditions with significant facial asymmetry, such as OAVS have a familial component. To this end we examined thirty-eight first degree relatives of individuals with OAVS. Thirteen (34.2%) had abnormally high asymmetry values based on one or both of the summary measures described above. These individuals were concentrated in just six of the twelve families comprising this preliminary study. These findings are consistent with other studies and support the theory that a genetic component exists in some cases of OAVS.

Program Nr: 832 from the 1999 ASHG Annual Meeting

Trisomy 7 mosaicism associated with carbohydrate-deficient glycoprotein syndrome and incontinentia pigmenti phenotype. *R. Jayoussi[1], R. Brill[1], H. Mandel[1,2], O. Lobel[1], I. Laeveski[1], Z. Galperin[1], R. Gershoni-Baruch[1,2]*. 1) Human Genetics, Rambam Medical Ctr, Haifa, Israel; 2) The Bruce Rappaport Faculty of Medicine, Technion, Institute of Technology, Haifa, Israel.

A girl with dysmorphic features, severe mental retardation and anomalous skin pigmentation associated with trisomy 7 and an unusual pattern of carbohydrate glycoprotein deficiency is described. Born at term to young, unrelated couple of Jewish Ashkenazi origin, following normal gestation and delivery. Birth weight was 2650g. (< 3rd centile) and OFC 34cm. (3rd centile). At two years of age she was referred for investigation because of slow psychomotor development. On clinical examination the following features were noted: high forehead, antimongolian slant, deep set eyes, a depressed nasal bridge, dysplastic low set ears, widely spaced inverted nipples, widespread irregular streaks and punctate lesions of depigmentation on trunk and all four limbs. Her lymphocyte karyotype was normal and she was given the diagnosis of incontinentia pigmenti. Anomalous pigmentation known to be correlated to chromosomal mosaicism or chimerism induced further evaluation and a skin fibroblast which was performed showed trisomy 7 mosaicism (46,XX/47,XX+7). FISH using a centromeric direct probe (Cytocell) confirmed the diagnosis. Extensive biochemical evaluation revealed an abnormal serum transferrin isoelectro-focusing pattern, consisting of an increased amount of disialotransferrin, reduced amounts of tetra, penta and hexasialotransferrin and a small amount of monosialotransferrin. Normal phosphomanomutase and phosphomanose isomerase activities in fibroblasts are consistent with the diagnosis of a previously undescribed variant of a carbohydrate-deficient glycoprotein syndrome. The association of the clinical biochemical and chromosomal abnormalities remains to be defined and clarified.

Program Nr: 833 from the 1999 ASHG Annual Meeting

Familial inheritance of a 5p14 deletion that results in a variable phenotype. *E.I. Johnson[1], R.C. Marinescu[1], H.H. Punnett[2], B. Tenenholz[3], J. Overhauser[1]*. 1) Dept. of Biochem/Mol Pharm, Thomas Jefferson University, Philadelphia, PA; 2) St. Christopher's Hospital for Children, Philadelphia, PA; 3) Penn State Geisinger Medical Center, Danville PA.

Deletions of 5p are normally associated with a diagnosis of cri-du-chat syndrome. Clinical features of this syndrome include a cat-like cry at birth, microcephaly, mental and developmental delay, growth delay and craniofacial features that include microcephaly, hypertelorism, and micrognathia. In 1986, a 3 generation family with 6 individuals having an interstitial deletion of 5p14 was described. All of the 6 individuals were completely asymptomatic. This suggested that a deletion of 5p14 is not associated with a clinical phenotype and does not contribute to the cri-du-chat syndrome phenotype. We report on a father and son who have an identical interstitial deletion of 5p14. Although the father is clinically and mentally normal, the son has significant clinical involvement including microcephaly, seizures, and global developmental delay. The location of the 5p14 deletion in the father and son was determined using fluorescent in situ hybridization and YAC clones that had been precisely mapped along 5p. Cells from a skin biopsy as well as blood were investigated in the father to rule out mosaicism. The extent of the deletion was compared with the deletion in the 3 generation family. The deletion in our father-son pair is smaller and entirely contained within the deletion previously described in a multigenerational family that lacks any clinical phenotype. This report demonstrates that the clinical outcome of a 5p14 deletion can vary even within the same family.

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Program Nr: 834 from the 1999 ASHG Annual Meeting

Graves disease in patients with microdeletion 22q11.2: Possible predisposition to autoimmune disorders. *H. Kawame[1], M. Adachi[2], K. Tachibana[2], M. Masuno[3], K. Kurosawa[4], Y. Ochiai[1], F. Ito[4], Y. Eto[4]*. 1) Dep of Pediatrics, Tokyo Metropolitan Kita Med & Rehabilitation Ctr, Tokyo; 2) Dep of Endocrinology, Kanagawa Children's Med Ctr, Yokohama; 3) Div of Medical Genetics, Kanagawa Children's Med Ctr, Yokohama; 4) Dep of Pediatrics, Jikei Univ. Schl of Med, Tokyo, Japan.

Phenotypes associated with the microdeletion 22q11.2 chromosme involve many systems. The list of clinical findings are quite long, including some endocrine findings, but etiological mechanism of those entire features have not been understood. Graves disease, the most common cause of thyrotoxicosis in childhood and adolescence, is an autoimmune disorder characterized by the production of stimulating antibodies to the thyrotrophic receptor. These antibodies lead diffuse goiter and hyperthyroidism.

We present three female patients with a microdeletion 22q11.2 and Graves disease. Graves disease was diagnosed at age 7, 10, and 17 years, respectively. Clinical presentation was typical for hyperthyroidism, such as weight loss, palpitations, polyuria, excessive sweating, and exophthalmos. All patients showed elevated serum levels of thyroid hormones in association of suppressed level of TSH. TSH-receptor antibodies were positive in all patients. Noteworthy all patients had thrombocytepenia and two showed elevated platelet-associated IgG (PA-IgG). All of them had hypocalcemia, two presented as late-onset hypoparathyroidism.

Recently, juvenile rheumatoid arthritis, autoimmune hemolytic anemia, and immune thrombocytepenia have been described in patients with a microdeletion 22q11.2. Our observation suggest that Graves disease may be part of the clinical spectrum associated with the 22q11.2 microdeletion syndrome. We also suggest that this syndrome is associated with predisposition to autoimmune disorders which explain some of the extremely variable phenotypes of this syndrome.

Program Nr: 835 from the 1999 ASHG Annual Meeting

Fryns Syndrome or Pallister-Killian (Tetrasomy 12p) Syndrome? The Importance of Delineation. *S.J. Kennedy, A. Chan, N. Al-Sannaa, I. Teshima, A.S. Teebi.* Clinical Genetics, Hospital for Sick Children, Toronto, Ontario, Canada.

Phenotypic overlap exists between autosomal recessive Fryns syndrome and Pallister-Killian syndrome (PKS). A definitive diagnosis of PKS can be made via detection of tetrasomy 12p on fibroblast culture. Two patients, who presented with eventration of the diaphragm, or diaphragmatic hernia, wide mouth, nail hypoplasia and other anomalies, were diagnosed initially as having Fryns syndrome. In patient 1, the presence of slightly coarse facies with bitemporal narrowing and upward slanting eyes along with a bifid left thumb and relatively large body size prompted us to request chromosome analysis on fibroblast culture. Tetrasomy 12p was confirmed on 24/25 metaphases examined. The patient's development was only slightly delayed and she did not show skin pigmentation problems which are unusual for patients with PKS. In patient 2, maternal serum screening was positive for trisomy 21, but amniocentesis was declined. The patient had profound hyotonia and the facies were coarse but not typical of PKS or any classical chromosomal syndrome. The patient's condition deteriorated rapidly and an early post mortem fibroblast culture confirmed the diagnosis of tetrasomy 12p. One of the patients originated from an ethnic group known to have high frequencies of autosomal recessive disorders, lending to the initial diagnosis of Fryns syndrome. This illustrates the importance of considering other diagnoses such as chromosomal disorders that may have lower recurrence risks. This also suggests that PKS should be considered in any case suspected to have Fryns syndrome for counselling purposes.

Program Nr: 836 from the 1999 ASHG Annual Meeting

Clinical features in a unique family with Autosomal Dominant Limb-Girdle Muscular Dystrophy and Paget Disease of Bone. *V.E. Kimonis, M. Kovach, R. Khardori, D.A Gelber.* Southern Illinois Univ Sch Med, Springfield, IL.

We have identified a large family with a unique phenotype: Autosomal Dominant Limb-Girdle-Muscular-Dystrophy (LGMD) associated with early onset Paget Disease of Bone (PDB). A large family with autosomal dominant amyotrophic lateral sclerosis and PDB first reported by Tucker et al. in 1982 (MIM 167320) bears some resemblance to this family. Clinical evaluation in eight affected individuals (5M, 3F), mean age 49 y. indicated that 6/8 had both LGMD/PDB with evidence of only PDB in the youngest individual age 34 y. The onset of bone pain leading to a diagnosis of PDB begins early at a mean age of 39.5 y. Muscle weakness and pain begins at a mean age of 41.5 y. Clinical findings include tenderness of the hip, shoulder and spine, muscular weakness and pain, wasting of the shoulder and pelvic girdle muscles, and reduced/absent deep-tendon reflexes. Muscle biopsy in 4 individuals revealed abnormal muscle with evidence of atrophic fibers, fibrosis and mild regeneration and degeneration. Consistent with PDB, X-rays show coarse trabeculation, and sclerosis, particularly of the pelvis, shoulder and skull bones. Bone biopsy in 1 female for suspected neoplasia confirmed PDB. Laboratory evaluation revealed elevated alkaline phosphatase (mean 250 U/L, normal 30-130) in all individuals except one male age 46 y. who only had LGMD. Interestingly this male had the highest creatine kinase level of 264 IU/L (mean of group 121.6, normal 20-260). High resolution karyotype was normal. A male died at age 50 y from muscular dystrophy and cardiomypathy. He also had insulin dependant diabetes mellitus and coronary artery disease. Affected individuals eventually become bedbound; several ancestors died prematurely in their fifties from LGMD/cardiomyopathy. The association of LGMD with Paget disease of bone appears to also be genetically unusual. Linkage analysis excluded the loci for dominant and recessive forms of LGMD, PDB and cardiomyopathy in the family. A genome search is in progress to identify the gene causing these two apparently unrelated disorders. The clinical and molecular data in this unique family will be presented.

Program Nr: 837 from the 1999 ASHG Annual Meeting

A mutation in the 5' non-HMG box region of the SRY gene in two patients with Turner syndrome and Y mosaicism. *S. Kofman-Alfaro[1], P. Canto[2], E. de la Chesnaye[2], A. Cervantes[1], M. Lopez[1], B. Chavez[3], F. Vilchis[3], E. Reyes[3], A. Ulloa[3], J.P. Mendez[2].* 1) Genetica, Hosp General de Mexico, Mexico, DF, Mexico; 2) UIM en Biologia del Desarrollo, CMN SXXI, IMSS, Mexico DF, Mexico; 3) Depto. de Biologia de la Reproduccion, INNSZ, Mexico, DF, Mexico.

Mutations of the SRY gene are the cause of sex reversal in approximately 10-15% of XY females with Y pure gonadal dysgenesis. Most of these mutations are located on the HMG box coding region of the gene. However, several mutations outside this region have also been reported. We performed molecular studies of the SRY gene in 3 subjects with Turner syndrome and mosaic karyotypes; patients 1 and 2 were 45,X/46,XY, while patient 3 was 45,X/46,X,+der(Y). In cases 1 and 3 a missense mutation, S18N, was identified in the 5'non-HMG box encoding region of the SRY gene in DNA extracted from peripheral blood and both gonadal streaks. This abnormality was not identified in the father and brother of patient 3 and it was not found in 75 normal males, excluding a common polymorphism. Recently, the same mutation was observed in a brazilian 46,XY female with partial gonadal dysgenesis, suggesting the existance of a hot spot in this region of the SRY gene. We consider that in our two patients the predominance of the 45,X cell line besides the existing mutation, prevented the development of testicular tissue. The magnitude in which the 45,X cell line or the SRY mutation affected the phenotype can not be ascertained.

Program Nr: 838 from the 1999 ASHG Annual Meeting

A new X-linked lissencephaly syndrome, Barry-Kravis and Israel syndrome: Lissencephaly with agenesis of corpus calloum in a Japanese family. *I. Kondo[1], J. Tohyama[2], T. Fukuda[1], K. Obata[1], H. Yamagata[1]*. 1) Dept of Hygiene, Ehime Univ. School of Medicine, Ehime 791-0295, Japan; 2) Dept. of Pediarics, Okinawa Child Development Center, Okinawa 904-2174, Japan.

Lissencephaly is one of the most severe brain malformations characterized by a smooth cerebral surface and agyria with or without pachygyria. More than 25 syndromes with lissencephaly have been described and divided into two distinct groups, type 1 (generalized lissencephaly), including the Miller-Dieker syndrome (MDS), and isolated lissencephaly sequence (LIS), and type II (hydrocephalic lissencephaly), involving the Fukuyama congenital muscular dystrophy. Most cases with lissencephaly are sporadic, and patients with MDS or LIS have a deletion within chromosome band 17p13.3. Some forms of lissencephaly, however, are familial, and one of the gene responsible for X-linked lissencephaly has been isolated and named as doublecortin. Lissencephaly is sometimes associated with agenesis of the corpus callosum. Almost all patients are sporadic, but two families with lissencephaly and agenesis of the corpus callosum have been reported, one family with X-linked and another one with autosomal recessive inheritance. We report a family that has two male infants with lissencephaly and agenesis of the corpus callosum. The clinical features in these patients were characterized by severe growth failure, psychomotor retardation and intractable seizures with the neonatal onset. 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. Magnetic resonance imaging (MRI) scan of the mother did not show a subcortical band heterotopia. On the basis of the clinical course and a family pedigree, our family seems to the second family with a X-linked lissencephaly syndrome described by Berry-Kravis and Israel. The main clinical characteristics of the Berry-Kravis syndrome are 1) intractable seizure, severe psychomotor retardation, and growth detraction, 2) agyria-pachygyria and agenesis of the corpus callosum in MRI scan, and 3) X-linked recessive inheritance.

Program Nr: 839 from the 1999 ASHG Annual Meeting

Caudal duplication sequence in a monozygotic twin. *H.Y. Kroes, J.C. Oosterwijk, A.J. van Essen.* Department of Medical Genetics, University of Groningen, Groningen, The Netherlands.

We present a girl with a spina bifida occulta, duplication of the distal spine including the sacrum, left double ureter, duplication of the vagina and cervix, and duplication of the distal colon with an ectopic anus and rudimentary perineal anus. She was one of a pair of monozygotic twins. Her sister had none of the above mentioned anomalies. This case is another example of the phenotype illustrated in the paper by Dominguez et al (1993), in which he presents six similar cases, and introduces the term caudal duplication syndrome. We propose the term caudal duplication sequence, as it is likely that one single event caused sequentially the duplications of the different involved organs. Monozygotic twinning is a known risk factor for anomalies in the caudal region like VATER association, caudal regression sequence, bladder exstrophy sequence, and sirenomelia. The occurrence of caudal duplication sequence in a monozygotic twin makes it very likely that the etiology is non-genetic, although a somatic mutation cannot be excluded.

Program Nr: 840 from the 1999 ASHG Annual Meeting

Identification of a novel common mutation in the connexin 26 gene (GJB2) among Japanese patients with childhood deafness. *T. Kudo[1,2], S. Kure[1], Y. Matsubara[1], K. Ikeda[2], T. Oshima[2], K. Watanabe[2], T. Kawase[2], T. Takasaka[2], K. Narisawa[1]*. 1) Department of Medical Genetics; 2) Department of Otorhinolaryngology-Head and Neck Surgery, Tohoku University School of Medicine, Sendai, Japan.

Mutations in the connexin 26 gene (GJB2), which encodes a gap-junction protein and is expressed in the inner ear, have been shown to be responsible for a major part of nonsyndromic hereditary prelingual (early-childhood) deafness in Caucasians. We have sequenced the GJB2 gene in 39 Japanese patients with childhood deafness (group 1), 39 Japanese patients with postlingual progressive bilateral sensorineural hearing loss (group 2) and 63 Japanese individuals with normal hearing (group 3). Three novel mutations were identified in group 1: a single nucleotide deletion (235delC), a 16 bp-deletion (176-191 del (16)) and a nonsense mutation (a408c) in five unrelated patients. The 235delC mutation was the most common, accounting for seven in ten mutant alleles. Screening of unrelated normal individuals for the three mutations indicated that the carrier frequency of the 235delC mutation was 2 in 203 in the Japanese population. No mutation was found in group 2 patients. We also identified two novel polymorphisms, a341g and t608c, as well as previously reported two polymorphisms (g79a and g109a). Genotyping with these four polymorphisms allowed normal Japanese alleles to be classified into seven haplotypes. All 235delC mutant alleles identified in four patients resided only on type 1 alleles, which would favor the hypothesis that the mutation has a single origin. The present study suggested that GJB2 mutations were responsible for approximately 13% (5 of 39) of prelingual deafness in Japan. The relatively high prevalence of the 235delC mutation and the availability of a simple DNA-diagnostic method would be useful in genetic counseling of prelingual deafness in Japan.

Program Nr: 841 from the 1999 ASHG Annual Meeting

Parental mosaicism of JAG1 mutations in families with Alagille syndrome. *A. Kujat, A. Röpke, I. Hansmann, I. Giannakudis.* Humangenetik, Martin-Luther-Universität, Halle, Germany.

The clinical features of the Alagille Syndrome (AGS) include chronic cholestasis, a typical face, heart, skeletal and ocular anomalies. The positionally cloned JAG1 gene within 20p12 has been found to be causally related to the disease. The analysis of pedigrees of AGS patients demonstrates highly variable expression. The clinical study of 33 AGS patients where mutations had been detected we found anomalies in liver 91%, heart 97%, face 97%, eye 87% and vertebrae 65%. Phenotypic differences between patients within same but also between different families carrying identical mutations were observed. In our screen (48 mutations were described) we observed mosaicism in four cases. In case 1, two sisters were affected by an insertion in exon 26 and was not observeable by sequencing the parent's DNA. By designing a primer that corresponds to the insertion the mutation was detected in the mother's DNA. In case 2 upon sequencing the father's DNA showed no signal corresponding to the mutation found in his offspring (R184H). Since this mutation creates a restriction site for PmlI we confirmed our observation by digestion, which resulted in mutation specific fragments in the patients and weaker in the father's DNA. In case 3 the clinically affected mother showed a mutation specific SSCP-shift of exon 5 with less than half of the intensity of the patient's band. Accordingly, sequence analysis of the respective exon of the mother disclosed a faint heterozygous signal representing a splice site substitution. In case 4 after microsatellite analysis using D20S1154 the patient's paternal allel showed a clear signal reduction appearing at less than half the dose as expected. The result was reproduceable with another pair of primer for the respective locus. We consider the signal reduction to be due to a deletion affecting about 25% of the lymphocytes, as judged from the signal intensity. Our results point to a possibly underestimated frequency of mosaicism in Alagille Syndrome occurring in up to 10% of the cases. Since mosaicism was detected in clinically mildly affected patients and in parents with no obvious anomaly, this has to be considered in genetic counselling.

Program Nr: 842 from the 1999 ASHG Annual Meeting

Intrafamilial correlation of clinical manifestations in neurofibromatosis 2 (NF2). *R.A. Kumar[1], M.E. Baser[2], D.G.R. Evans[3], A. Wallace[3], V.F. Mautner[4], L. Kluwe[5], H. Joe[1], J.M. Friedman[1], G. Rouleau[6].* 1) Dep't Medical Genetics, UBC, Canada; 2) L.A., USA; 3) St. Mary's Hosp, Manchester, UK; 4) Klin. Nord Ochsenzoll, Hamburg, Germany; 5) Univ Hamburg, Germany; 6) McGill Univ, Quebec, Canada.

Anecdotal evidence suggests that familial correlations exist in disease presentation in patients with neurofibromatosis 2 (NF2). We analyzed intrafamilial correlations in age at onset of first symptom, age at hearing loss and number of intracranial meningiomas in 223 patients from 55 families with NF2. The correlation coefficients and their corresponding 95% confidence intervals are shown in the table.

	Age at onset	Age at hearing loss	No of meningiomas
All patients	0.32 (0.19, 0.54)	0.37 (0.16, 0.59)	0.48 (0.33, 0.68)
Excluding probands	0.40 (0.22, 0.60)	0.58 (0.35, 0.77)	0.56 (0.39, 0.75)

Consideration of the mutation types separately was limited by small sample sizes, but the correlation coefficients were generally higher in 8 families with germ-line nonsense or frameshift mutations and in 9 families with missense mutations than in 10 families with with splice-site mutations. This is consistent with previous reports of phenotypic heterogeneity in patients with splice-site mutations. The most striking finding for a specific mutation type was the high intrafamilal correlation of age at hearing loss for patients with missense mutations (r = 0.83; 95% CI, 0.51-0.99). This may reflect the occurrence of a milder overall phenotype among patients with germ-line missense NF2 mutations and consequent larger family sizes. We conclude that some NF2 clinical manifestations exhibit significant intrafamilial correlations and that allelic heterogeneity is an important determinant of disease severity in NF2.

We thank the Acoustic Neuroma Association of Canada and the BC Medical Services Foundation for their support.

Program Nr: 843 from the 1999 ASHG Annual Meeting

Familial and Sporadic acromegaly: A distinct clinical entity. *W.W. Lam[1], S. Orme[3], G. Taylor[2], P. Belchetz[3], C.E. Chu[1]*. 1) Dept Clinical Genetics, St James's Hosp, Leeds; 2) Yorkshire Regional DNA Laboratory, St James University Hospital, Leeds; 3))Dept Endocrinology, The General Infirmary at Leeds.

Acromegaly is a chronic disorder resulting from excessive secretion of growth hormone. It may occur as an isolated phenomenon or as part of the Carney complex or Multiple Endocrine Neoplasia type 1(MEN1). Isolated acromegaly is a rare disorder with an incidence of around 1 in 30,000 and is usually considered to be sporadic but familial cases have been recognised. Some acromegalic families have been found to have mutations in the MENIN gene.The aim of the study was to investigate the incidence of familial isolated acromegaly in a population of acromegalics attending a regional endocrine clinic and the possibility of germline mutations in the MENIN gene in patients with isolated acromegaly and whether this is part of the MEN1 spectrum.30 kindreds (total of 257 subjects; including 32 patients with isolated acromegaly with 2 sib pairs, 60 parents, 93 siblings and 72 children) were studied for evidence of clinical features of acromegaly. This was performed by 2 experienced endocrinologist working independently, viewing serial photographs and biochemical investigations of suspected cases.22 acromegalic patients, including 3 sib pairs (one extra-regional), were then investigated for germline mutations by genomic sequencing of the coding region and all intron-exon boundaries of the MEN1 gene using Big-Dye terminators and the ABI 377 semi-automated sequencer.No new cases of acromegaly were identified but 2 sib pairs in such a cohort was highly significant (O/E ratio 396.8; $p<0.0001$). No mutations were detected in either familial or sporadic patients. This suggests that familial and sporadic isolated acromegaly are not part of the MEN1 phenotype. Combined with studies which link isolated acromegaly to chromosome 11, this would indicate that a possible second tumour suppressor gene may be present.

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Program Nr: 844 from the 1999 ASHG Annual Meeting

Adverse phenotypes associated with gonosomal aneuploidy. *R.R. Lebel[1], R.L. Brown[2].* 1) Genetics Services, Glen Ellyn, IL; 2) Univ Colorado Health Sci Center, Grad Program in Genetic Counseling, Denver, CO.

Gonosomal aneuploidy (45,X; 47,XXX; 47,XYY; various mosaic states) may have a cumulative incidence greater than that of trisomy 21, and may pose a greater challenge to genetic counseling. Prospective studies generally show highly variable phenotypes, but seldom serious malformations or developmental delays (typical in 45,X or high-order multiples such as tetrasomy). We report 15 sex chromosome aneuploidies other than 45,X and 47,XXY: 7 cases of 47,XXX, 3 of 47,XYY and 5 mosaics. Eight were diagnosed prenatally for indications of maternal age (6), delayed fertility (1) or thick fetal nuchal skin fold (1); this last proved to be associated with 47,XXX and Tetralogy of Fallot. One woman referred for maternal age had ventriculomegaly found by ultrasound at time of amniocentesis, which revealed 47,XXX. Five postnatal diagnoses were made after referral for malformations (47,XXX with cleft palate; X aneuploid mosaicism with aniridia; 47,XYY with craniosynostosis and cryptorchidism), secondary amenorrhea, or recurrent miscarriages (1). One case of 47,XYY was made at autopsy of a 19 week miscarriage with urogenital anomalies. Mosaic 45,X/47,XYY was found in a miscarried twin pregnancy. Though the etiologic significance of these aneuploid states is indeterminate, and the association with a seriously abnormal phenotype might be coincidental, it is important to report these observations in view of what is known of the potential importance of other aneuploid states, including other gonosomal aneuploidies such as 45,X and 47,XXY. Genetic counseling is complex when findings such as these are at hand, either prenatally or postnatally.

Program Nr: 845 from the 1999 ASHG Annual Meeting

Febrile convulsions, ataxia, developmental delay and overgrowth-a new MR syndrome. *D. Lev[1,3], N. Watemberg[2,3], T. Lerman-Sagie[2,3]*. 1) Institute of Medical Genetics, Wolfson Medical Center, Holon, Israel; 2) Pediatric Neurology Unit; 3) Metabolic Neuro-Genetic Clinic.

We describe a new syndrome consisting of recurrent complicated febrile convulsions, developmental delay, ataxia and overgrowth in three unrelated girls. The three girls, aged 3 to 4 years were all born to healthy non-consanguineous parents and have normal siblings. Their birth weight was appropriate for gestational age. They are not dysmorphic and have normal head circumference. Development was delayed: they walked with ataxic gate after the age of 2 years and spoke at 3 years. Their growth charts are remarkably alike: they initially had a normal growth curve and around 24 months of age they started to gain weight excessively. They all continue to suffer from complicated febrile seizures, which started before 12 months of age and are resistant to anticonvulsive medications. Metabolic evaluation was normal. They have normal MRI and normal EEG. Fragile X syndrome and Prader-Willy syndrome was ruled out. We suggest this is a new MR/Overgrowth syndrome that should be considered in children with recurrent febrile convulsions, developmental delay and obesity.

Program Nr: 846 from the 1999 ASHG Annual Meeting

Eleven years old Arab girl with trisomy 13. *A.A. Abdulaal, Sr., S.S. Al Arrayed, F.A. Ali, H. Sanad.* Genetic Dep., Salmaniya Medical complex., Manama., Bahrain.

A case of trisomy 13,confirmed by G banded chromosome analysis, in an eleven years old Bahraini girl. There is no cytogenetic evidence for mosaicism in the propositus or her parents. banding analysis showed a complete extra chromosome 13.\parThis female baby was born on 7.5.88 by assisted breech to a 37-years old gravida 8 para 7 mother,who had uneventful pregnancy and delivery.\parBirth weight was 2.9kg,(10th centile)birth length47.5cm(3rd centile)head circumference 31.5cm(below 3rd centile)and apgar scores 9and 9 at 1 and 5 minutes respectively.\parThe following congenital abnormalities were noted at birth:Microcephaly,high arched palate, micophthalmia,scalpdefect with abnormal hair whorls, haemangiomata of the face, overlapping of fingersleft hnds simian crease,abnormal feet,all other system were normal.\par screening for congenital infection was done,which was negative.Skull Xrays and skull ultrasound were normal.Renal ultrasound ,echocardiogram were normal. ocular ultrasound confirmaed bilateral microphthalmia.\parAt two months of age,patients develop recurrent grand mal epilepsy for which she was hospitalized many times.EEG shows a diffues brain dysfunction with multiple epileptiform activity.\parShe suffered severe developmental and mental retardation and was bedridden all her life.\parAdmitted recently for convulsion at age of 11 years, all her growth parameter were below 3rd centile. .MRI brain show gross atrophic changes in the brain.

Program Nr: 847 from the 1999 ASHG Annual Meeting

Trisomy / monosomy of the N-MYC region in sibs arising from a unique maternal karyotype. *J. Ahmad[1], S. Herath[1], T. Barnhart[1], J. Andeweg[1], S. Baldinger[2], T.M. Sutton[3], E. Bendelstenzel[3], C.A. Curtis[1], R.R. Higgins[1]*. 1) Allina Cytogenetics Laboratory, Abbott Northwestern Hospital, Minneapolis, MN; 2) Genetic Counseling Director, Abbott Northwestern Hospital; 3) Children's Hospitals and Clinics, Minneapolis, MN.

Cytogenetic studies were requested on a one day-old infant with pulmonary atresia and ventricular septal wall defect. Other phenotypic findings included: patent ductus arteriosis, hypoplastic left ventricle and mitral valve, posteriorly rotated ears and hypoplastic nails. Chromosome analysis revealed a 46,XX karyotype with a small interstitial deletion in one chromosome 2 involving G-band region 2p24.1-2p25 that included the N-MYC proto-oncogene. Parental chromosomes were done. The father's karyotype was 46,XY. The mother's karyotype included the deleted chromosome 2 previously identified in the child but was balanced by the insertion of that chromosome 2 segment into one chromosome 1 at G-band region 1p34.2 [46,XX,ins(1;2)(p34.2;p24.1p25)]. Cytogenetic results were confirmed by FISH using probes specific for the 2p subtelomeric sequences and the N-MYC proto-oncogene. Cytogenetic analysis of the 3 year-old brother of the proband showed he inherited the der(1) from the mother, but not the der(2) [46,XY,der(1)ins(1;2)(p34.2;p24.1p25) mat]. This karyotype results in trisomy for the 2p24.1-2p25 region and N-MYC. The brother is noted as also having hypoplastic left heart syndrome with significant aortic arch obstruction. A recent chest x-ray has demonstrated pulmonary edema with significant cardiomegaly. He is developmentally delayed with an estimated developmental quotient of 2 and 2 1/2 years of age. His height is 86.4 cm (3rd percentile) with a weight of 13.8 kg (25th percentile). He has had a tracheostomy and was hospitalized for several episodes of pneumonia. He continues to be trached and is ventilated, has subglottic stenosis and left vocal cord paresis. Four patients with a deletion of the N-MYC region have been summarized by Saal et al. (1996), but this appears to be the first report of a patient trisomic for a small chromosomal segment encompassing the N-MYC proto-oncogene.

Program Nr: 848 from the 1999 ASHG Annual Meeting

Duplication Xq in a severely affected male with 46,XY,der(21) t(X, 21)(q26; p13) identified by chromosome microdissection. *M. Akiyama[1], H. Ohashi[2], T. Tohma[2], H. Ohata[1], A. Shishikura[1], I. Miyata[1], N. Usui[1], Y. Eto[1], H. Kawame[3]*. 1) Dept. of Pediatrics, Jikei Univ. School of Medicine, Tokyo; 2) Div. of Medical Genetics, Saitama Children's Med. Center, Saitama; 3) Dept. of Pediatrics, Tokyo Metropolitan Kita Med.& Rehabilitation Center, Tokyo, Japan.

Functional disomy of partial Xq chromosome with unbalanced X-autosome translocation is extremely rare. Male patients with functional X disomies usually have severe phenotypic outcome, although female patients show variable phenotypes. We report a male infant with MCA/MR and a duplication Xq26-qter on derivative chromosome 21 identified by chromosome microdissection. The proband was 9-month-old male infant. He was born at 33 weeks to a 30 years old, healthy mother. He had severe prenatal-onset growth retardation, microcephaly, dysmorphic facial features, cryptochidism, and left multidysplastic kidney. Neurologically, he had remarkable hypotonia, infantile spasms, severe developmental delay, and feeding difficulty. Brain CT revealed agenesis of corpus callosum. His blood chromosome analysis revealed 46, XY, add (21) (p13). Chromosome analysis of his parents was denied. In order to define the extra material on der (21), chromosome microdissection was performed. The dissected DNA was amplified and labelled using degenerated oligonucleotide primed polymerase chain reaction (DOP-PCR). The labelled DNA was reverse painted to the patient's cells and his karyotype was concluded as 46, XY, der (21) t (X; 21) (q26; p13). Functional Xq disomy was supported by the absence of XIST on der (21). Duplication Xq26-qter attached to Xp has been described in only one family previously. To our knowledge, this is the first case to be described with this cytogenetic abnormality due to unbalanced translocation defined by microdissection.

Program Nr: 849 from the 1999 ASHG Annual Meeting

Clinical, Endocrine and Cytogenetic study of 141 pediatric patients with ambiguous genitalia. *A. Al-Mutair, M.A. Iqbal, A. Ashwal, N. Sakati.* Cytogenetics/Molecular Genetic, King Faisal Specialist Hospita, Riyadh, Saudi Arabia.

In the present study, we report our data on 141 newborn patients referred for karyotyping with a primary indication of ambiguous genitalia. Chromosome analysis and hormonal evaluations were performed according to routine laboratory methods.

Out of 141 patients, 22 (15.7%) were excluded because the criteria for ambiguous genitalia did not fit in these patients. The remaining 119 patients with sexual ambiguity were due to: a) endocrine defects – 52.4%; b) non-endocrine defects – 47.8%. In the group with endocrine defects, congenital adrenal hyperplasia was the underlying cause in 41 patients; the karyotype of these patients was 46, XX (39 cases) and 46, XY (2 cases). The remaining 22 cases in this group showed 46, XY karyotype and consisted of vanishing testicular syndrome (6 cases); Leydig cell hypoplasia (5 cases); androgen insensitivity syndrome (5 cases); 5 alpha reductase deficiency (3 cases); gonadal dysgenesis (1 case); para hypopitutirism (one case) and true hermophrodite (1 case).

In the non-endocrine defect group, hypospiadias was seen in 11 cases all of these had a 46, XY karyotypes. Cloacal anomaly was present in 10 patients, six of these were 46, XY and 4 were 46, XX. Congenital local genital malformations were observed in ten cases (9 cases = 46, XY; 1 case = 46 XX). Nineteen cases were due to metabolic defects or associated with dysmorphic features. 7 cases were classified as idopathic (6 cases = 46, XY; 1 case = 46, XX).

Thirteen patients were wrongly referred as having ambiguous genitalia (most probably by a general pediatrician). All of these cases were later found to be normal by the pediatric endocrinologist.

Approximately 10% patients were raised phenotypically different than their chromosomal sex. Our data emphasizes the importance of rapid clinical and laboratory evaluation of newborns with ambiguous genitalia to eliminate life threatening medical and psychological problems.

Program Nr: 850 from the 1999 ASHG Annual Meeting

Phenotype of patient with pure partial trisomy 2p (p23®pter). *M. Al-Saffar[1,4], E. Lemyre[2,4], R. Koenekoop[3,4], A.M.V. Duncan[2,4], V.M. Der Kaloustian[1,4].* 1) F. Clarke Fraser Clinical Genetics Unit, Departments of Pediatrics and Human Genetics; 2) Department of Pathology; 3) Department of Ophthalmology; 4) Montreal Children's Hospital and McGill University, Montreal, Quebec, Canada.

We present a 7-month-old female with the karyotype 46,XX,der(13)(p23;p11.2).ish der(13)(wcp2+). Painting confirmed that the additional segment on 13p was of chromosome 2 origin, resulting in trisomy 2p23®2pter. Parental karyotypes were normal. The child had a prominent forehead with a flat hemangioma, depressed nasal bridge, protruding tongue, and posteriorly angulated ears.

Ophthalmological examination revealed esotropia with poor abduction of the right eye, bilateral high myopia (-5.50 D), retinal hypopigmentation, foveal hypoplasia and striking left optic nerve hypoplasia. Pectus excavatum and a protruding abdomen with diastasis recti were also observed. There was generalized hypotonia. Fine and gross motor developments were at the one-month age level. Abdominal U/S was normal. A voiding cystourethrogram indicated grade II reflux on the left and grade III-IV reflux on the right side. An EEG revealed epileptiform discharges. CT scan of the brain showed decreased white matter, but MRI was normal.

Twelve cases reported in the literature have partial trisomy of 2p23®2pter. These are inherited as unbalanced translocation and associated with partial deletion of various chromosomes (Bender et al., 1969; Carrier,1975; Magenis et al., 1975; Francke and Jones, 1976; Cassidy et al., 1977; Neu et al., 1979; Rosenfeld et al., 1982; Fineman et al., 1983; and Laurie et al., 1995). As a result of the associated partial monosomies of diverse chromosomes, these patients have different phenotypes. There are no reports of *de novo* direct duplication of 2p23®2pter. Our patient represents the only case of pure partial trisomy of this segment.

Program Nr: 851 from the 1999 ASHG Annual Meeting

The behavior of molecular cytogenetic parameters in parents of Down Syndrome offsprings. *A. Amiel, O. Reish, I. Kedar, M. Fejgin.* Genetics Unit, Meir General Hosp, Kfar-Saba, Israel.

We attempted to demonstrate a loss of replication control and thereby centromere dysfunction leading to predisposition to nondisjunction in 10 couples with a Down Syndrome (DS) offspring and in elderly women. One color FISH was applied to interphase nuclei (lymphocytes). Replication pattern of two pairs of allels, RB-1 and 21q22 were studied and the rate of aneuploidy was estimated using two alpha satellite probes of chromosomes 8 and 18.

	ASYNCRONOUS REPLICATION*		HYPER	PLOIDY *
patients	21q22	RB-1	8 alphasatel	18 alphasatel
DS mothers n=9	18+/- 1.3 **	17 +/- 1.1 **	3.7+/- 0.6 **	4+/-0.6 **
elderlies n=4	17+/-1.0 **	15+/-1.8 **	4+/-0.1 **	4.6+/-1.0 **
controls n=13	11+/-1.0	8+/-0.9	1.5+/-0.2	1.5+/-0.3

* mean 0f the proportion of cells with asynchrony or hyperploidy ** $p<0.01$ Asynchronous replication pattern (SD Pattern) was significantly higher in elderly women and in mothers of DS (both young and old mothers) compared to the control group($p<0.01$) in both loci studied. The rate of hyperploidy (three or more signals) with the two alpha sattelite loci was also significantly higher in these groups ($p<0.01$). Our results suggest the existence of an association between replication timing and the rate of non-disjunction. A higher rate of asynchronization and aneuploidy was found in older women and in mothers of DS offsprings irrespective of age. These findings may reflect a predisposition for neiotic non-disjunction in these women.

Program Nr: 852 from the 1999 ASHG Annual Meeting

Towards the clinical validation of Spectral Karyotyping (SKY). *A. Anguiano, V. Sulcova, M. Ayad, A. Sbeiti, A. Fraser, S. Wang, L. Edwards, K. Reddy, B. White.* Cytogenetics Dept, Quest Diag, Nichols Inst, San Juan Capist, CA.

Spectral Karyotyping (SKY) is an interferometer based method that allows the distinctive identification of all 24 human chromosomes by color after the hybridization with chromosomal DNA libraries that are each combinatorially labeled with as much as five fluorochromes. A number of reports have shown that SKY is useful to identify chromosomal exchanges and to characterize marker and ring chromosomes. In this study we aimed to test the performance of SKY in a cytogenetics reference laboratory. The vendor's protocol was followed. We studied cytogenetic preparations from peripheral blood of 20 normal subjects (10 males and 10 females). We also studied 30 cytogenetic preparations from selected abnormal cases having chromosomal abnormalities such as translocations, additional genetic material in certain chromosomes, and marker or ring chromosomes. The abnormal cases were representative of prenatal, pediatric, reproductive investigation, products of conception, and hematologic malignancy (peripheral blood or bone marrow) cases. The study of chromosomes from the peripheral blood of normal subjects yielded a 99.2% accuracy of automatic classification of chromosomes. All SKY results of constitutional abnormalities were confirmed with GTG banding and/or whole chromosome painting; some were also confirmed by locus specific FISH probes. One constitutional case and a significant number of hematologic malignancy cases revealed cryptic rearrangements. Confirmatory chromosome painting was not performed for cases with hematologic malignancies. While we were able to consistently identify origin of small markers and ring chromosomes, the lower limit of resolution of SKY has yet to be defined. However, our results demonstrate that the method was highly accurate and informative in the cases selected for analysis. Additional studies are now in progress to further evaluate and establish the role of SKY in our clinical laboratory practice.

Program Nr: 853 from the 1999 ASHG Annual Meeting

Significant hypotonia and developmental delay: Hallmarks of a terminal deletion of chromosome 2q. *H.H. Ardinger[1], D.L. Persons[1], R.E. Lutz[2], M.A. Beattie[2].* 1) Pediatrics, University of Kansas Medical Center, Kansas City, KS; 2) Pediatrics, University of Kansas School of Medicine-Wichita, Wichita, KS.

Patients with a terminal deletion of the long arm of chromosome 2 have been rarely reported. We present the clinical findings of a 5 year old girl whose deletion was not initially detected on a high resolution analysis. A second analysis coupled with a FISH test using a 2q telomere probe confirmed the deletion of 2q37-qter.

The patient presented with a history of pronounced hypotonia that did not affect infant feeding, but which delayed ambulation until age 4 years. She has had surgery for coarctation of the aorta and eyelid ptosis. She is followed for bicuspid aortic valve and aortic stenosis, a seizure disorder and severe developmental and cognitive delay especially in the area of language. Growth has been normal. She has a number of minor dysmorphic facial features in addition to a narow thorax with low nipple placement, small labia majora, proximally placed thumbs, long slender fingers, flat narrow feet, thin lower legs and hyperextensible joints.

There are 4 patients reported in the literature with a terminal deletion of 2q involving band 2q37 in two, 2q36->qter in one and 2q35->qter in one. Comparing our patient to the previous reports, the 5 patients are all noted to have hypotonia and developmental delay. Dysmorphic features common to all are not particularly distinctive and include frontal bossing, long eyelashes and micrognathia. Our patient is the only one to have a heart defect.

Because of the lack of a specific facial phenotype or particular structural anomalies, and because the deletion may be difficult to detect, it is possible that this diagnosis is being missed. High resolution chromosome analysis with special attention to 2qter and FISH studies with a 2q telomere probe are recommended for any undiagnosed patient presenting with significant hypotonia (walking delayed beyond 2 years) and developmental delay even if unaccompanied by other anomalies.

Program Nr: 854 from the 1999 ASHG Annual Meeting

Microarray based Detection of Single Copy Sequence Changes in Genomic DNA. *Y. Bao, D. Che, N. Lermer, J. Shi, A. Prokhorova, U. Müller.* Advanced Technology, Vysis, Inc., Downers Grove, IL.

The Vysis GenoSensor microarray system consists in a unique chromium coated chip surface, multi-color fluorescent hybridization chemistry, and a wide-field non-scanning CCD based imaging system. This system has been successfully applied to the detection of sequence amplifications in tumor tissues, whereby genomic DNA was extracted from tumor cells and labeled with a green fluorophor, then hybridized to a microarray in the presence of normal human reference DNA (labeled red) and Cot-1 DNA (to suppress repeat sequences). The microarray contains 3 spots for each genetic locus, which for this application consisted mostly in oncogenes that have been shown to be potentially amplified in tumor tissues. A mixture of tumor DNAs with known amplifications has been used to measure the performance of this system, and to demonstrate reproducible detection of 2 fold amplifications with >95% confidence on each chip. For applications to prenatal diagnosis the reliable detection of single copy gene deletions or additions is essential. As proof of principle we have carried out hybridizations with normal female (green) and normal male (red) total human DNA. Target spots containing the androgen receptor (X chromosome) revealed a Green/Red ratio significantly higher than the ratios of all other loci (>99% confidence level), suggesting sufficient sensitivity for detection of single copy deletions. Using the DNA from three patients with various cytogenetic abnormalities (deletions and/or unbalanced translocations) we have been equally successful in detecting trisomic or monosomic regions. Supported by the National Institute of Standards and Technology ATP Award 94-05-0021.

Program Nr: 855 from the 1999 ASHG Annual Meeting

Molecular cytogenetic characterization of a familial translocation (1;4) with a breakpoint telomeric to the most distal subtelomeric single copy probe known on chromosome 4. *I. Bartels[1], S.K. Bohlander[1], Y. Mehraein[2], M.R. Speicher[3], S. Uhrig[3], B. Zoll[1].* 1) Institute of Human Genetics, University of Goettingen, Goettingen, Ger; 2) Institute of Human Genetics, Medical University of Hannover, Germany; 3) Institute of Anthropology and Human Genetics, Muenchen, Germany.

A 28 year old pregnant woman presented in our genetic counselling unit. She had two pregnancies: one was terminated because the male fetus had a hydrocephalus, the other was a missed abortion at 8 weeks of gestation. Her lymphocyte karyotype revealed additional G-band negative material at band 4p16.3. Hybridization with a probe for 4p16.3 (Wolf-Hirschhorn) showed that the breakpoint was distal to this locus. 24-colour multiplex-FISH analysis demonstrated material not originating from chromosome 4, but the additional segment was too small to be identified by this method. A subtelomeric probe from 4p also hybridized to the der(4) chromosome. The most likely region of origin of additional material was thought to be the short arm of chromosome 1. A subtelomeric PAC clone from 1p hybridized only to one 1p, the other signal was present on the der(4)p. Thus the additional material at 4p originated from 1p. This was not visible on the 500-band level. The fetus was shown to carry both derivative chromosomes. In this family, applying molecular cytogenetic methods is essential to differentiate balanced and unbalanced carriers of the translocation. To our knowledge this is the first example of an apparently balanced but non-reciprocal translocation.

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Program Nr: 856 from the 1999 ASHG Annual Meeting

Primary ovarian failure associated to a 46,XX,t(6;18)(p23;q22) balanced reciprocal translocation. Possible new autosomal genes involved in ovarian development. *E.F. Bastos[1,3], J.C. Llerena Jr.[1,2], J.C. Cabral de Almeida[1,2]*. 1) Instituto de Biofísica CCF, Univ. Fed. do Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil; 2) Centro de Genética Medica - Instituto Fernandes Figueira - FIOCRUZ - Rio de Janeiro, Brazil; 3) Instituto de Biociências - Universidade do Grande Rio - Prof. José de Souza Herdy - Rio de Janeiro, Brazil.

Primary ovarian failure has heterogeneous causes. Among genetic causes, X monosomy and Xp11/Xq13 deletions are among the principal causes associated with primary or premature ovarian failure. We describe a 22 year old woman with primary amenorrhea, no dysmorphies or neuro-developmental anomalies, absence of secundary sexual development, hypergonadotrophic hypogonadism and biopsy of both streak gonads with scarcity of primordial follicles. The cytogenetic analysis by G- band in lymphocytes showed a translocation between chromosomes 6 and 18 with breakpoints at 6p23 and 18q22. The investigation using FISH with a whole chromosome painting probe for chromosome 18 (wcp18)confirmed the reciprocity of this translocation characterizing the karyotype as 46,XX,t(6;18)(p23;q22).ish(6;18)(wcp18+;wcp18+). The parents were not available for investigation. The presence of an autosomal balanced translocation in this case points to new autosomal sites in the search for genes associated to ovarian development.

Program Nr: 857 from the 1999 ASHG Annual Meeting

Duplication 1q32->q44 and Multiple Congenital Anomalies. *J. Bayani[1], J. Xu[2,3], V. Freeman[2,3], J. Watts[4], P.A. Smith[5], J. Squire[1], M.J.M. Nowaczyk[2,3,4].* 1) Cellular and Molecular Biology, Princess Margaret Hospital, Toronto, Ontario, Canada; 2) Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Canada; 3) Hamilton Regional Laboratory Medicine Program, Hamilton, Canada; 4) Department of Pediatrics , McMaster University, Hamilton, Canada; 5) Department of Obstetrics & Gynecology, McMaster University, Hamilton, Canada.

We report a prenatal detection of de novo direct 1q duplication. A prenatal referral was made because of abnormal ultrasound findings of cerebral ventriculomegaly and bilateral choroid plexus cysts 24 weeks gestation. The amniocentesis showed an abnormal male karyotype with a duplication of the long arm of chromosome 1, 46,XY,dup(1)(q32q44). Duplication of this segment was subsequently confirmed by G-banding of peripheral blood lymphocytes and comparative genomic hybridization using DNA extracted from blood. Parental karyotypes were apparently normal. The baby was born at 37 weeks of gestation with dysmorphic features. His head circumference was 35.5 cm (90 centile), weight was 2600 g (10-25th centile), and length was 47 cm (25th centile). The anterior fontanel measured 6 x 5.5 cm (> +2SD), and there was a posteriorly sloping forehead. The ears were round in shape with unfolded helices. He had hypertelorism, short, downward-slanting palpebral fissures and a high-arched and narrow palate. Both of his feet were large with overriding second and third toes. Head CT scan showed prominent posterior horns of the lateral ventricles but normal third and fourth ventricles. The radiographic skeletal survey showed 11 pair of ribs. Sonographic evaluation of the abdomen showed left hydronephrosis. He also had a medium sized patent duct arteriosus, pre-ductal shelf of the aortic arch and tricuspid regurgitation. This is the 2nd report of 'pure' duplication of 1q32-q44. Flatz and Fintasch (1979) reported the first case in a 6 week old boy with pointed chin, beaked nose, midface hypoplasia, enlarged skull and fontanelles, unilateral cryptorchidism and hypospadias. Our case will help establish the correlation of this chromosome region and the clinical features.

Program Nr: 858 from the 1999 ASHG Annual Meeting

Chromosome 8p23.1 duplication: is there an association with a clinical phenotype? *M.L. Begleiter, H.A. Cooper, L.M. Pasztor, M.G. Butler.* Sect Medical Gen/Molec Med, The Children's Mercy Hospitals & Clinics, Kansas City, MO.

A 5 8/12 year old boy was referred for evaluation of developmental delay and mild dysmorphic features. The family history was significant for clinical depression in the mother and bipolar illness in the father. The child had attention deficit hyperactivity disorder, bipolar illness, myopia, a coarse facial appearance, an abnormally placed hair whorl and prominent ears with a malformed low-set left ear. He had significant speech delay and late acquisition of motor skills. His karyotype was 46,XY,dup(8)(p23.lp23.1). By FISH analysis whole chromosome 8 painting probes hybridized to both chromosome 8s in their entirety. There was no evidence for the fragile X DNA mutation. Studies of other family members identified the dup(8p) in the child's mother, both his half siblings (through his mother) and in his maternal grandfather. None of these individuals had developmental delay or dysmorphia.

Duplication of 8p23.1 has been previously documented in 14 families. In only three of these families were any clinical stigmata described. In one family, a girl was identified with short stature (her mother, who was of normal height also had the duplication). In another family, an eighteen-month-old boy with developmental delay was found to have a de novo duplication. In a third family, two sisters with minor dysmorphia and normal development and their nondysmorphic mother were shown to have the 8p duplication. Thus, most individuals with the same euchromatic duplication seen in our patient have been described as phenotypically normal. It has been suggested that duplication 8p23.1 is a cytogenetic anomaly of no known established clinical significance and that the few cases of phenotypically abnormal individuals who carry this duplication represent bias of ascertainment. Our family adds additional evidence to support this impression.

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Program Nr: 859 from the 1999 ASHG Annual Meeting

Gene expression profile analyses in Roberts Syndrome. *J.W. Belmont[1], A. VanHooser[2], L.R. Adam[2], L.G. Shaffer[1], B.R. Brinkley[2], O.A. Cabello[2].* 1) Dept Mol & Human Genetics, Baylor Col Med, Houston, TX; 2) Dept Cell Biology, Baylor Col Med, Houston, TX.

Roberts syndrome (RS) is a rare AR disorder with variable phenotype. Clinical features include tetraphocomelia, growth and mental retardation, genitourinary abnormalities, and increased rate of malignancy. Cell from the RS+ complementation group exhibit premature centromere separation in metaphase. We probed the integrity of chromatin remodeling pathways in RS+ cells. Posttranslational modifications of histones associated with mitotic condensation were assessed by immunofluorescence. In mitotic RS fibroblasts, chromosomes stained intensely with antibody against tetraacetylated H4, indicating lack of deacetylation compared to control. These results suggest a defect in mitosis-specific deacetylation of histone H4. HDAC has been shown to interact with MeCP2 and deacetylation may cooperatively mediate transcriptional repression. However, we detected no disturbance in methylation of *SNRPN*, a locus known to be regulated by methylation status, in RS+ cells. We used a filter cDNA array with approximately 18,000 known cDNAs and ESTs (GDA1.3) to analyze the transcription profile of mutant and normal lymphoblast cell lines. In two independent replicates, we detected asymmetric global distribution of expressed loci in control cells vs RS+. We restricted quantitative analysis to transcripts in the 90%ile for signal intensity i.e. correlating with standard analytical methods like Northern blot. After correction for global mean signal there was excellent concordance of most individual transcript hybridization intensities. However, we found that the distribution of the log(Control/Roberts) signal intensity was significantly skewed toward the Control (skewness 0.83±.05). 71% of transcripts (175/245) exhibiting discordant expression (>10-fold) were under-expressed in the Roberts cells. There is not yet extensive experience with expression array data in mammalian mutants and a broader interpretation of these data will depend on the specific mRNAs affected by the RS+ mutation. Evaluation of the repressed transcripts may advance understanding of RS as a model of chromatin structure disorders.

Program Nr: 860 from the 1999 ASHG Annual Meeting

Another cause for an extra marker chromosome: unequal segregation of the products of a maternal fission chromosome 19. *C. Booth, L. Jazmines, D. Rita.* Pediatrics, Lutheran General Hosp, Park Ridge, IL.

Chromosome studies were performed on the products of conception from a first trimester loss of a 30-year-old woman with a history of repeated miscarriages. 47 chromosomes were found, including an extra unsatellited marker. Parental chromosomes were studied. The father's were normal. The mother had 47 chromosomes with centric fission of one chromosome 19 [47,XX,-19,+fis(19p)(p10),+fis(19q)(q10)]. The fetal marker was identified as the fission chromosome 19p. Centric fusion produces Robertsonian translocations, some of the most common rearrangements in man. Centric fission is rare but, when present, often leads to reproductive wastage and/or failure. Parental chromosome studies should always be performed when a marker chromosome is found in fetal material.

Program Nr: 861 from the 1999 ASHG Annual Meeting

Two distinctively different phenotypes of 2q37 terminal deletion: Location of a gene on telomeric 2q37 mimicking Albright hereditary osteodystrophy/ pseudohypoparathyroidism. *B.R. Braddock[1], A.E. Shrimpton[2], C.S. Stein[1,2], J.J. Hoo[1]*. 1) Dept. of Pediatrics, Div. of Genetics, SUNY Health Science Center, Syracuse, NY; 2) Dept. of Pathology, SUNY Health Science Center, Syracuse, NY.

We report a 17 year old male with clinical features suggestive of Albright hereditary osteodystrophy (AHO)/pseudohypoparathyroidism (PHPT). He has mild to moderate mental retardation, stature below the 5th percentile, a round face with mildly upslanting palpebral fissures, myopia, multiple nevi on his upper trunk, abdominal obesity and delayed puberty with Tanner stage 1 external genitalia. He also presents with hypoplastic 3rd and 4th metacarpals and a short 4th metatarsal, bilaterally, and flat feet. He has a history of focal seizures up until age 7. Cytogenetic studies revealed an apparently terminal deletion of the long arm of chromosome 2 [46,XY,del(2)(q37.1)]. The break appears to occur distally in q37.1 since a portion of the band is still clearly detectable at the end of the chromosome. Molecular delineation will be carried out to clarify the breakpoint.

Terminal 2q37 deletion has been frequently reported. Review of the literature revealed that there are two distinctively different phenotypes. The majority of cases are associated with moderate to severe mental retardation, nonspecific dysmorphic features, and moderate to severe growth retardation. It is postulated that most of these cases are due to an interstitial deletion of the proximal portion of the 2q37 band. The second recognized phenotype from at least 7 cases in the literature (Wilson, L.C. et al., 1995; Power, M.M. et al., 1997) is characterized by milder mental retardation and a phenotype resembling AHO/PHPT. While classic AHO/PHPT is caused by a mutation in the alpha subunit of a G protein gene (GNAS1 gene) on 20q13.2, Power et al. suggested that a vasoactive intestinal peptide receptor gene on the telomeric 2q37 band is likely a candidate gene for 2q37 deletion cases resembling AHO/PHPT. The phenotypic and cytogenetic findings in our patient are consistent with this hypothesis and molecular delineation will provide further verification.

Program Nr: 862 from the 1999 ASHG Annual Meeting

The human homolog of the rat phospholipase C beta1 gene (PLCB1) maps at 20p12. *G. Calabrese[1], E. Morizio[1], D. Peruzzi[2], L. Stuppia[1], D. Fantasia[1], V. Gatta[1], G. Sabatino[1], L. Cocco[2], G. Palka[1], F.A. Manzoli[2].* 1) Dip Scienze Biomediche/sez Genetica Medica, Univ G. d'Annunzio, Chieti; Italy; 2) Cellular Signalling Lab, Ist Anatomia UN, Universita' di Bologna, Italy.

The hydrolysis of inositol lipid by specific phospholipase C (PLC) constitutes a key step of a signal transduction pathway that ultimately regulates a number of cellular processes such as proliferation, secretion, and neural activity. Among the 3 groups of PLC characterized in mammalians (beta, gamma, and delta), PLC beta consists of at least 4 isoforms of which type beta1 is of interest because of its localization within the nucleus as well as at the plasma membrane. We previously reported the mapping of the Plc beta1 in rat at chromosome band 3q34-35. With the aim of identifying its human homolog, we selected a clone, named plc1, from a human fetal brain cDNA library using the rat cDNA as a probe. The alignement of the 5.1 kb plc1 probe sequence with the 3.5 kb rat full length cDNA showed a 89% homology. Northern blotting experiments on human and rat total brain RNA displayed two similar transcripts of 7.2 kb and 5.4 kb with clone plc1 and rat full length clone, respectively. The human cDNA clone was then used in FISH experiments on human metaphases. In 50 cells signals were observed on chromosome 20 at band p12 in one or both homologs. This localization confirmed the synteny between rat chromosome 3 and human chromosome 20 and provided a novel homolog locus between bands q35 in rat and p12 in man. Such a synteny is also supported by co-localization of another PLC beta isoform, i.e. beta4, mapped at 20p12 which showed a 11% homology with clone plc1. Band 20p12 has been recently reported amplified and/or deleted in different solid tumors such as head and neck, lung, duodenal, and bladder cancers. Thus, the observation of activation of the nuclear Plc beta1 during cellular proliferation and its down-regulation during differentiation in normal and neoplastic cells in vitro further suggests the involvement of PLC beta1 in neoplasms. These evidences are also supported by the frequent rearrangement in rat tumors of band 3q34-35 where the rat Plcb1 maps.

Program Nr: 863 from the 1999 ASHG Annual Meeting

Delayed membranous ossification of the cranium associated with familial translocation (2;3)(p15;q12). *C.B. Cargile[1, 4], I. McIntosh[2], M. Clough[2], R. Yaghmai[1], B.K. Goodman[3, 4], G.H. Thomas[1, 4], M.T Geraghty[1]*. 1) Department of Pediatrics; 2) Medicine; 3) Obstetrics and Gynecology, Johns Hopkins School of Medicine, Baltimore, MD; 4) Kennedy Krieger Institute, Baltimore, MD.

The relationship of delayed membranous cranial ossification to cranium bifidum and parietal foramina syndromes is unclear. We now report a family with delayed cranial membranous ossification that segregates with a reciprocal translocation between chromosomes 2 and 3. The proband was born at 38 weeks by cesarian section with vacuum suction due to fetal brachycardia. The baby was noted to have low set ears, proptosis and the skull was not palpable. A radiographic survey of the skeleton showed markedly decreased ossification of the cranial bones and no other skeletal abnormalities. Serum calcium, phosphorous, alkaline phosphatase and thyroid function tests were normal. The mother and maternal grandmother have brachycephaly, hypertelorism and history of a soft skull. Chromosome analysis of peripheral blood from the proband showed 46,XY,t(2;3)(p15;q12). The proband, mother and grandmother carry the same reciprocal translocation, while the mothers normal siblings have a normal karyotype. The gene for the a1 chain of type VIII collagen, COL8A1, which maps to chromosomal region 3q12 and is expressed in the calvarial bones of newborn mice, was investigated as a candidate gene for delayed membranous cranial ossification. FISH analysis with COL8A1 phage clone showed signal close to, but not disrupted by, the familial translocation breakpoint. Southern blot analysis of COL8A1 showed no evidence of altered gene structure. We identified BAC clones from chromosomes 2 and 3 that map proximally and distally to the translocation breakpoint. This is the second report of familial delayed membranous cranial ossification and the first time it has been associated with a familial reciprocal translocation.

Program Nr: 864 from the 1999 ASHG Annual Meeting

Chromosome constitution studies in buccal smear samples by fluorescent in situ hybridization (FISH). *V. Catala[1], E. Cuatrecasas[1], T. Vendrell[2], M. Crespo[1], J. Colomer[1], A. Plaja[2], C. Mediano[2], A. Seres-Santamaria[1].* 1) Prenatal Genetics. Casanova 153 08036 Barcelona, Spain; 2) Unitat Gentica Hospital Materno-Infantil Vall d'Hebron. Barcelona.

Buccal smear analysis have been used for interphase analysis of X and Y chromosomes for over 30 years using the Barr body test for determination of X chromatin and the fluorescent Y-chromatin test. These methotds are not specific and sensitive enough for detection of mosaicism, because the X-chromatin can be identifies in the 20-40% of the cells in normal females, and the Y-chromatin is only observed in about the 50% of the normal male cells. The study of autosomes had not been possible at that moment. The introduction of non-isotopic fluorescent in situ hybridization (FISH) technology using specific probes for each chromosome allows interphase analysis of aneuploidies and sex determination. We have analyzed the exfoliated epithelial cells in 3 controls and 5 clinical cases (2 newborn infants cytogenetically normals with suspected Pallister-Killian S., 2 dismorphic newborn infants with c-anaphases in the cytogenetic study, and a 19-years-old female cytogenetically XY after blood culture due to a bone marrow transplantation from a male donnor with suspected Turner Syndrome.

Program Nr: 865 from the 1999 ASHG Annual Meeting

PCR-FISH-PRINS analysis of marker chromosomes in 8 patients with Turner phenotype. *A. Cervantes[1], M. Lopez[1,2], R. Guevara Yanez[3], N. Monroy[1], M. Aguinaga[1], H. Valdez[4], C. Sierra[4], S. Canun[4], J. Guizar[3], C. Navarrete[3], G. Zafra[5], F. Salamanca[3], S. Kofman[1].* 1) Genetica, Hospital General Mexico SSa, F Medicina UNAN, Mexico DF; 2) S Biologicos UAM-X, Mexico DF; 3) UIMGH Hospital Pediatria CMNSXXI IMSS, Mexico DF; 4) S Genetica Hospital Gea Gonzalez SSa, Mexico DF; 5) S Genetica Hospital Espanol,Mexico DF.

The presence of Y-derived material in patients with Turner phenotype is correlated with a high risk of developing gonadoblastoma and dysgerminoma, requiring preventive removal of the dysgenetic gonads. Therefore, it is clinically relevant to determine the chromosomal origin of marker chromosomes that cannot be identified by conventional banding techniques. Also, their identification is important for establishing phenotype-karyotype correlations. We describe the identification of marker chromosomes in 8 patients with Turner phenotype by a combination of PCR, FISH and PRINS methodology in DNA and cell cultures from blood lymphocytes. In all cases, initial karyotypes were 45,X/46,X,+mar; six of the marker chromosomes were rings of different sizes, 3 being particularly small. PCR analysis for Y-sequences (PABY, SRY, ZFY, Ycen and Yqh) were negative for the 6 patients with ring chromosomes and positive in the rest. PRINS were done with Xcen and Ycen primers in all patients, demonstrating the X origin of all the ring chromosomes and the Y origin of the remaining 2 marker chromosomes, one being dicentric. FISH with Xcen and Yqh probes was performed in the patients with Y material, showing that one has very little heterochromatine while the other marker has signals on both sides. Taken together, data shows that the latter chromosome is pseudodicentric with a breakpoint in Yp11.3. Phenotype-karyotype correlations could not be establish since mosaicism of highly variable nature can exhibit an unpredictable outcome. None of the patients had mental retardation, including those with tiny X-chromosomes. Replication studies demonstrated the inactivation of all the X-derived ring chromosomes, suggesting the presence of XIC. We discuss the advantages of the combined use of PCR, PRINS and FISH over the use of single or conventional techniques.

Program Nr: 866 from the 1999 ASHG Annual Meeting

Ring chromosome 16 in a normal female. *K.E. Chandler, K. Turnbull, K.L. Gaunt.* University Department of Medical Genetics and Regional Genetics Service, St Mary's Hospital, Manchester, UK.

Ring chromosome 16 in a normal female. Chandler, KE (1); Turnbull, K (1); Gaunt, KL (1) (1) University Department of Medical Genetics and Regional Genetics Service, St Marys Hospital, Hathersage Road, Manchester, M13 0JH A 34 year old woman was referred for genetic counseling after an in utero death at 28 weeks following 7 years of infertility. Cytogenetic investigations revealed her karyotype to be 46,XX, r(16)(p13;p3q24) in 186/200 cells examined. 8/200 cells had a 45,XX,-16,karyotype, 4/200 cells had a double ring 16 and 1/200 had a small ring 16. Only 1/200 cells had a normal 46,XX karyotype. Skin chromosomes showed ring 16 karyotype with a similar low level of mosaicism. Fetal material was not available for karyotyping and post mortem was unremarkable save for growth retardation. A subsequent spontaneous miscarriage at 8 weeks gestation was found to have a mosaic ring 16 karyotype with a large number of abnormal ring derivatives. To date, only 7 cases of ring (16) have been reported in the literature. Normal cell lines and 45,XX,-16 cell lines were found in most of these, unlike our case where a low level of mosaicism was found. In all cases the patient had significant learning difficulties and mild dysmorphic features. Most of them had growth retardation.Our patient was of above average intellect and of normal appearance and stature. Apart from infertility, she has had no health problems. Ring formation by means of telomere/telomere fusion is proposed as no loss of genetic material could be seen on cytogenetic analysis. This may explain our patients normal appearance and intelligence. The structural instability of a ring chromosome gives rise to continuous generation of aneuploid cells. Such cells are likely to be lost at subsequent cell divisions. This could explain her recurrent fetal loss and infertility. Several complex counseling issues have been raised by this case.The couple have opted to be assessed for pre-implantation genetic diagnosis.

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Program Nr: 867 from the 1999 ASHG Annual Meeting

X CHROMOSOME ABNORMALITIES : REPORT OF 14 CASES. *V. CHARPENEL[1], L. TELVI[1], R. ION[1], J.L. CHAUSSAIN[2]*. 1) LABORATOIRE DE CYTOGENETIQUE, HOPITAL ST VINCENT DE PAUL, PARIS, FRANCE; 2) SERVICE D'ENDOCRINOLOGIE PEDIATRIQUE, HOPITAL ST VINCENT DE PAUL, PARIS, FRANCE.

We describe 14 cases with X chromosome abnormalities as Xp- (3 cases), Xp+ (1 case), Xq- (2 cases), Xq+ (1 case), X-Y translocation (1 case) X-Autosome translocations (3 cases) and complex X chromosome abnormalities (3 cases). Chromosome analysis required RHG, CBG, QFQ, RTBG bandings, and completed in some cases by FISH analysis of the X chromosome, Y chromosome, XIST gene or translocated autosomal chromosomes. The inactivation of normal or abnormal X chromosome was analyzed by RTBG banding. The growth retardation, mental retardation, dysmorphia, Turner syndrome features and the presence or absence of gonadal dysgenesis were analyzed and discussed. The growth retardation is variable from the average to -8SD and depending of the chromosomal breakpoints. The mental retardation is present in all 3 cases with X-Autosome translocations. In other cases mental retardation is rare and found only in 2 patients. The expression of dysmorphia and Turner syndrome features depend on the localisation of the breakpoints and the percentage of cells with inactivated abnormal X chromosome.The presence or absence of gonadal dysgenesis depend also of the localisation of the breakpoints. The gonadal dysgenesis was found in the majority of cases (11 out 14 cases). These findings should be helpfull in genetic counselling.

Program Nr: 868 from the 1999 ASHG Annual Meeting

Nonrandom rearrangements of 6p in malignant hematological disorders. *Z. Chen, B. Issa, L.J. Brothman, M. Hendrickson, D. Button, A.R. Brothman.* Dept Pediatrics/Cytogenetics, Univ Utah Sch Med, Salt Lake City, UT.

Anomalies of chromosome 6, often present as 6q-, have been associated with lymphoid disorders, especially acute lymphocytic leukemia and lymphoma. However, it is very uncommon to observe anomalies of 6p in malignant hematological disorders (MHD). A literature review shows 6p anomalies as a sole change in only three cases and as an evolutionary anomaly in 36 cases of MHD. By using conventional cytogenetics and FISH with chromosome-microdissection probes specific for 6p21 and 6p25, we observed five patients with myeloid malignancies and two patients with lymphoid malignancies to have 6p anomalies. Possible biologic characteristics of such aberrations in MHD can be divided into three groups: The first group presents with 6p anomalies as a sole change in myeloid malignancies with only two cases reported, including one case with del(6)(p23) and one present case with ins(6)(q23p23p25)identified by FISH only; both cases have the same breakpoint (p23) involved, suggesting a nonrandom relationship between the 6p23 breakpoint and myeloid malignancies. The second group presents with 6p anomalies as a sole change in lymphoid malignancies with three cases reported, including one with del(6)(p21p23), one with del(6)(p21), and one present case with del(6)(p21). A potential tumor suppressor gene, located in 6p21-p23, may be specifically associated with the pathogenesis of some lymphoid malignancies. The third group has 6p anomalies, in addition to other known primary changes, present in 36 cases of myeloid or lymphoid disorders, including five cases from our series. Deletions involving 6p21, 6p22, or 6p23 have been observed in both myeloid and lymphoid disorders. The primary changes often present with the 6p anomalies include t(9;22), 5q-, 20q-, 7q-, -7, +8 and 11q23-related changes in myeloid disorders and 9p-, +3, +7, t(8;14), and other 14q32-related abnormalities in lymphoid disorders. It appears that 6p anomalies may be breakpoint-specific disease-related when presented solely, or may represent an evolutionary event when appearing with other primary changes. The present data provide cogent information for further molecular characterization of 6p anomalies in MHD.

Program Nr: 869 from the 1999 ASHG Annual Meeting

HC Forum ® : an international database and a Web site dedicated to structural abnormalities of chromosomes;.
O. Cohen, M.A. Mermet, J. Demongeot. Genetic Lab/TIMC/UMR CNRS 5525, Grenoble Univ Medical Sch,La Tronche, France.

Structural abnormalities represent many difficulties for risk assessment. On the other hand, they represent a powerful tool for research on the genome.

Since 1988, we have developed an international database, which now includes familial data from 3.500 reciprocal translocations and 1.000 pericentric inversions carriers. From this database, we have developed a Web site, named HC Forum® (Human Cytogenetics Forum)° , which offers different tools for medical genetics and to research workers:

- Assistance for diagnosis whith a schematic view of rearranged chromosomes, according to the ISCN
- Assistance for genetic counselling with risk assessment specific to each abnormality for guiding the strategy of prenatal diagnosis or assisted procreation and many other usefull information: pachyten diagram, list of the potential imbalances with a prediction of their viability, retrospective data, references.
- Cartographic applications that show the distribution of the chromosomal breakpoints and of the viable partial trisomic and monosomic regions of the genome. Interactive screens allow finding information specific to breakpoints or imbalances such as references or contacts to find biological material.
- A data submission process with an interactive pedigree drawing and an availability to send an image of the karyotype.
- A forum for discussion about the submitted cases.
- A link to other Human genetic Web sites.

More than 200 geneticists from more than 25 different worldwide countries are already users. Finally, this Web site, which needs a preliminary registration, represents a link between the diagnosis centers where many biological of interest exist, and the research teams which need specific biological materials but don't know where they are available.

http://HCForum.imag.fr.

Program Nr: 870 from the 1999 ASHG Annual Meeting

Mild learning disabilities and anomalies associated with monosomy/trisomy of 11q in a 3-generation family carrying an insertional translocation. *L.A. Correia[1], A. Nadel[1,2], W.A. Miller[3], B.R. Braddock[3], A. Milunsky[4], H.E. Wyandt[4], M. Sandstrom[5]*. 1) Maternal Fetal Medicine, The Women's Health Center of the North Shore, Danvers, MA; 2) Obstetrics and Gynecology, Brigham and Women's Hospital, Boston, MA; 3) Perinatal Diagnosis Center, Inc., Lexington, MA; 4) Center for Human Genetics, Boston University School of Medicine, Boston, MA; 5) Pathology, Brigham and Women's Hospital, Boston, MA.

The index case, a 39-year-old G5P1SAB2TAB2 female, has a deletion of 11q, i.e. 46,XX,del(11)(q14q21)pat. She presented at 6 weeks gestation with a history of being a slow learner and having a third toe curled under the fourth unilaterally. She graduated from high school and attended college for a short time. She was diagnosed with ADD as an adult, although formal IQ testing was not performed. The family history is significant. A brother with learning disabilities has the same deletion. His daughter reportedly has speech delay but has not been karyotyped. A second brother with learning disabilities and behavioral problems has not been karyotyped. A paternal first cousin has a karyotype of 46,XX,der(20)ins(20;11)(q12;q14q21). She has learning disabilities, low set ears and a missing incisor. She had two prior pregnancies studied by amniocentesis at ages 29 and 30. Both pregnancies showed karyotypes identical to her own; the first, a female, was therapeutically aborted; the second, a male, is now three years old. The karyotype of the father of the index patient is apparently balanced with an insertion of a piece of the long arm of chromosome 11 into the long arm of chromosome 20, i.e. 46,XY,ins(20;11)(q13.1;q14q21). Fluorescent in situ hybridization using a whole chromosome paint (WCP11, Oncor) confirmed this translocation in the father and the cousin. Probes for Cyclin D at 11q13 and MLL at 11q23 indicate that the breakpoints on chromosome 11 are telomeric to Cyclin D and centromeric to MLL. An amniocentesis of the patient's current pregnancy yields a normal, 46,XX, fetal karyotype. Discussion will include the segregation of the familial translocation and unbalanced products through three generations.

Program Nr: 871 from the 1999 ASHG Annual Meeting

Mosaic tetraploidy in a healthy woman with recurrent pregnancy loss. *J.M. Cowan, M.S. Murray, P.G. Wheeler, D.W. Bianchi.* Division of Genetics,Department of Pediatrics, Tufts University School of Medicine, Boston, MA.

Background: There are relatively few case reports in the literature of individuals with mosaic tetraploidy. In all cases, patients have been ascertained by mental retardation, abnormal phenotype, or congenital anomalies. Our patient (TC), a 37 yo G9P3Sab4Tab1 healthy employed woman, presented for genetic evaluation following the loss of triploid conceptus (68,XXY).

Methods: Standard peripheral blood karyotype analysis was performed on two occasions. A skin biopsy was obtained for fibroblast karyotype analysis. Interphase FISH using the alphoid probe for chromosome 7 was used to determine ploidy in the samples. The frequency of tetraploid cells in TC's blood samples was compared with the frequency in blood samples from 3 controls who presented with a history of infertility or pregnancy loss.

Results: Tetraploid cells were observed in preparations of TC's first peripheral blood sample, and were also present in the repeat blood specimen and the skin fibroblast cultures. TC had 1-2% tetraploid cells in her 2 blood samples, while the control patients had 0% in each of 4 cultures per patient. The frequency of tetraploid cells in the skin sample was 6% by FISH analysis.

Conclusions: There are 2 interpretations of the results in this case: either the patient has a diploid karyotype and error-prone cell division or she is a mosaic 92,XXXX/46,XX. There are problems with both conclusions. FISH demonstrated division of the tetraploid cells and it might be expected that failure of cell division would result in octaploid cells. No octaploid cells were seen. If the patient is a mosaic for tetraploidy, she is much more mildly affected than the previously reported cases. The loss of the conceptus with triploidy, however, suggests that a percentage of TC's oocytes are tetraploid, and become diploid during meiosis.

Program Nr: 872 from the 1999 ASHG Annual Meeting

Search for a gene disrupted in a patient with acrodysostosis and a t(4;6)(q12;p23). *J.G. Dauwerse[1], B. de Vries[2], C.H. Wouters[2], D.J.M. Peters[1], M.H. Breuning[1].* 1) Dept of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands; 2) Clinical Genetics Center Rotterdam, Rotterdam, The Netherlands.

The index patient is a mentally normal 31 year-old male with height 1.40m (-6SD), arm span 1.26m and normal head circumference (54.5 cm = 25th centile) with a flat face, depressed nasal bridge, broad nasal root and anteverted nostrils. He had extreme brachydactyly of both hands and, to a lesser degree, of the feet. The aorta was sclerotic with insufficiency of the aortic valves. X-ray investigation showed acrodysostosis with relatively short ulna suggesting either acrodysostosis (MIM 101800) or a mild form of acromesomelic dysplasia. Both non-consanguineous parents and 5 sibs had normal postures. A de-novo 46,XY,t(4;6)(q12;p23) was found.
In order to find the gene(s) disrupted by the t(4;6) we began by mapping the chromosome 4 breakpoint more precisely. FISH with YAC clones selected from the SHGC chromosome 4 YAC map was performed on metaphase slides of the patient. Out of 40 YACs tested, five YACs turned out to span the chromosome 4 breakpoint. Using Alu-PCR fragments from the smallest YAC (330Kb) two PAC clones were isolated, which FISH signals were split by the translocation. Using the PACs, cosmids could be isolated and a restriction map of the region was constructed; however, cosmids spanning the breakpoint could not be isolated. Using the same FISH mapping approach a YAC (1.5Mb) spanning the chromosome 6 translocation breakpoint has also been identified. Currently various techniques are applied to identify genes in the cloned chromosome 4 region. Within the cosmid contig mapping distal to the translocation breakpoint we could place the PDGFRA-KIT genecluster. A fragment close to the breakpoint, showing cross-species conservation is currently being evaluated.

Program Nr: 873 from the 1999 ASHG Annual Meeting

An unusual case of a mosaic Turner syndrome variant with a tiny r(X) and a rare partial proximal duplication of 1q. *A.J. Dawson[1,2], D.E. Wickstrom[1], D. Riordan[1], S. Cardwell[3], R. Casey[3].* 1) Cytogenetics Laboratory, HSC, Winnipeg, MB, Canada; 2) Depts. Human Genetics and Pediatrics, University of Manitoba, Winnipeg, MB, Canada; 3) Division of Medical Genetics, Royal University Hospital, Sakatoon, SK, Canada.

A 7 yr old female with global cognitive impairment and delayed motor and mental development as well as significant hyperactivity and impulsivity was referred to the Genetics clinic. Height and weight were at the 5th% while head circumference was at the 50th%. Dysmorphic features included bluish sclera, low set and slightly posteriorly rotated auricles and a narrow palate with marked overbite. There was no significant family history. Chromosome analysis showed an unbalanced, mosaic female karyotype consisting of three cell lines: 46,X,+r[46]/45,X[37]/45,X,dup(1)(q11q21.3)[17].ish r(X)(DXZ1+,XIST+). The first cell line, present in 46% of cells, was missing a normal X chromosome and had a small ring chromosome which was identified as an active pericentromeric derivative of the X chromosome containing the XIST locus. The second cell line, present in 37% of cells, had a classic Turner karyotype with a missing X chromosome. The third cell line, present in the remaining 17% of cells, was also missing an X chromosome and, in addition, had a direct duplication of chromosome 1 from bands q11 to q21.3. The tiny r(X) syndrome with the karyotype 46,X,r(X)/45,X is known to have functional X disomy for the region encompassed by the r(X) and is generally associated with a severe phenotype of physical and mental handicap. The presence of the XIST gene is consistent with an active X chromosome. The clinical phenotype of the tiny r(X) syndrome in our patient is obviously further influenced by mosaicism for the dup(1). Few cases of duplication of the proximal portion of chromosome 1 have been reported. Of these, the duplication either was present in all cells or involved different band regions so that a direct comparison is difficult. However, the lower percentage of mosaicism for the dup(1) in our patient would suggest a milder influence on the clinical phenotype. The parental karyotypes are normal.

Program Nr: 874 from the 1999 ASHG Annual Meeting

Case of supernumerary der22,T(11/22), born to a mother mosaic for the 11/22 translocation. *M. Descartes, A. Carroll.* Department of Pedicatris, Uviv. of AL. at Birmingham, AL.

Our patient was the first child born to a 21 year old female at term by SVD. Her birth weight was 3.2kg (50th), her length was 53cm (50th), and her FOC measured 34cm (25th). She was noted at birth to have dysmorphic features and CHD(coartation of the aorta and AV canal). Her physical examination was pertinent for: malformed ears with preauricular pits and tags, fronto orbital hypoplasia, scarce eyebrows, flat nasal bridge, epicanthic folds, micrognathia, facial asymmetry, hypoplasia of the labia majora, anteriorly placed anus. Peripheral blood cytogenetic analysis revealed a 47,XX,+der(22)t(11;22)(q23;q11.2) karyotype. Maternal chromosome analysis of peripheral blood on 2 different occasions revealed that she was a mosaic carrier of the 11;22 translocation, mos46,XX,t(11;22)(q23;q11.2)/46,xx. Chromosome studies of the maternal grandparents revealed normal karyotypes for both. The t(11;22) is thought to be one of the most common reciprocal translocations in man. While most reported cases have been familial, at least 3 instances are known where the balanced t(11;22) occurred "de novo" in a carrier parent. Mosaicism for any balanced translocation is very rare and we are unaware of any previous report of mosaicism for the balanced t(11;22). We presume that our proband's mother's cells were initially normal, and that a very early somatic event led to the translocation that was present in approximately 50% of her PHA stimulated leukocytes. Chimerism seems unlikely, but has not been ruled out. The recurrence risk we quoted the mother was the same (2-6%) as that listed in the literature for non-mosaic carrier females.

Program Nr: 875 from the 1999 ASHG Annual Meeting

Tetrasomy Y by structural rearrangement: A patient with two isodicentric Y chromosomes and global psychomotor delay. *M. DesGroseilliers[1,3], E. Lemyre[2,3], L. Dallaire[2,3], N. Lemieux[1,3].* 1) Department of Pathology and Cell Biology, University of Montreal, Montreal; 2) Service of Medical Genetics, Ste-Justine Hospital, Montreal; 3) Research Center, Ste-Justine Hospital, Montreal, Quebec, Canada.

Poly-Y karyotypes -except for 47,XYY- are rare events in humans. Y chromosome tetrasomy, for instance, has only been reported 9 times - one of which was by structural rearrangement. We present a 2-year-and-4-month-old boy who was referred for cytogenetic assessment because of global psychomotor delay. The GTG and CBG banded karyotypes on PHA-stimulated lymphocytes showed two cell populations, one of which contained two identical isodicentric Y chromosomes in 93% of metaphases analyzed and a 45,X cell line (7%). This was confirmed by FISH with probes DYZ3 (specific for the centromeric region of Y chromosome), 91H4.5 (recognizing Yp11.2) and DYZ1 (recognizing Y heterochromatin in Yq12). The breakpoint is near the end of the heterochromatic region. Therefore, the karyotype is mos 47,X,idic(Y)(q12)x2[123]/45,X[9]. This is the second time that such a karyotype is reported. Clinical features included speech delay, short stature, brachycephaly, large ears, bilateral epicanthal folds, hypertelorism, delayed teeth eruption, bilateral radio-ulnar synostosis, normal external genitalia and impulsive behavior. The father had normal phenotype and karyotype. A review of the poly-Y karyotypes is presented, as well as the most likely mechanism by which these two isodicentric Y chromosomes occurred. Most patients with Y chromosome tetrasomy exhibit some degree of mental retardation, various skeletal abnormalities and facial dysmorphism. Nevertheless, the correlation between the karyotype and the phenotype is not yet well defined since few cases have been reported. This case report will be helpful in defining the phenotypic range of the tetrasomy Y patients. (*This research was supported by grants from Réseau de Médecine Génétique Appliquée-FRSQ).*

Program Nr: 876 from the 1999 ASHG Annual Meeting

CYTOGENETIC EXPRESSION OF FRAXA AND IT'S RELATIONSHIP WITH SPEECH DELAY. *M. Diaz Garcia, G. Garca Sanchez, F. Huesca Hernandez, J.E. Dominguez-Aburto L.* Instituto Nacional de la Comunicacion Humana SSa. Mexico, City.

In Mexico the studies in the Fragile X-Syndrome or FRAXA has been realized in people with mental retardation and not in individuals with normal intelligence (IQ) or boderline (70-80) and speech delay. For this reason we decide to search the presence cytogenetic of FRAXA in a population of 50 individuals with speech delay and IQ >70 to determine if the presence of FRAXA associates with one or more speech delay. The 50 patients were selected through the consultation of Genetics, to them realized their clinical history and diagnosis of psychologicaaal, speech, audiologic, etc. For the cytogenetic study the chromosomal preparations was realized from lymphocyte culture, using 3 media for the induction of FRAXA, 300 metaphases was analyzed per patient. All patients were diagnosed with speech delay: perserveration, echolalia and articulation and omissio errors. The most patients, presented characteristics of common behabior: emotional lability, short lapses of attetion and low tolerance to the frustration, 17 patients presented alterations in one or both ears, 34 presented other alterations phenotypic common to the syndrome. All the patients had antecedent hereditary of speech delay. In the cytogenetic study 45 patients were negative FRAXA and 5 positive FRAXA with different percentages in the expression. However, with the application of the citogenetic techniques, we cannot identify the carrier FRAXA and possibly in some patients the alteration it is masked, whereby is requieres the use of methodology applied in Molecular Biology to detect carrier though of number CGG repeat, that are in Xq27.3.

Program Nr: 877 from the 1999 ASHG Annual Meeting

Hydrocephalus and microcephalus associated with a satellited 6q in a family segregating a t(6;21) and a paracentric inversion in 9q. *T.C. Drumheller, J. Low, S. Katz, R. Vandergon, T. Treisman, C.J. Curry.* Dept Cytogenetics, Genetic Medicine, Valley Children's Hosp/UCSF, Madera, CA.

X-linked hydrocephalus was suspected in a male proband and his sister's male child, both diagnosed with hydrocephalus and moderate mental retardation. However, mutational analysis of L1CAM was negative. Following the birth of the sister's second child, a daughter with severe microcephaly, chromosome studies were repeated.

Chromosome analysis of all three severely-affected family members revealed a chromosome 6 with a satellited long arm and deletion of 6q25.3®qter. A balanced translocation between the long arm of chromosome 6 and the short arm of chromosome 21 was found in the proband's father and twin sisters. Another sister, who is short and obese with borderline normal intelligence, was found to have the unbalanced derivative chromosome 21 with trisomy for 6q25.3®qter. A subtle paracentric inversion in the long arm of chromosome 9, seen in both affected and non-affected family members, appears to be a familial variant having no clinical significance. The findings in this family suggest that genes critical for normal brain development lie within the region of 6q25.3®qter and that haploinsufficiency of these genes causes severe abnormalities with significant mental impairment. This case further underscores the importance of repeating cytogenetic studies when the clinical geneticist suspects a chromosome abnormality. FISH analysis as well as high-resolution cytogenetic analysis were required to characterize both rearrangements segregating in this interesting family.

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Program Nr: 878 from the 1999 ASHG Annual Meeting

Pachygyria and polymicrogyria, cranio-facial dysmorphism and atrio-ventricular canal resulting from a duplication of the proximal region of chromosome 11q (11q11-11q12). *O. Dupuy[1], I. Husson[2], J. Wirth[3], J. Wiss[1], C. Nessmann[1], P. Evrard[2], P. Eydoux[1]*. 1) Cytogenetics, Hopital Robert Debre, Paris, France; 2) Neurology, Hopital Robert Debre, Paris, France; 3) Max Planck Institute for Molecular Genetics, Berlin, Germany.

Very few cases of imbalance of the paracentromeric region of chromosome 11 have been described. We report a patient with delayed development, seizures, cranio-facial dysmorphism, and a congenital heart disease, with a duplication of the centromere and paracentromeric region of chromosome 11. This male patient was born at 41 weeks; oligohydramnios, intra-uterine growth retardation and a small biparietal diameter were noted during the pregancy. An amniocentesis was performed and did not demonstrate any chromosomal aberration. Clinical abnormalities included unusual cranio-facial features (microcephaly, facial asymetry, upslanted palpebral fissures, dysplasia of the right ear, high arched palate), seizures, sleep disturbance, axial hypotonia with limb hypertonia. An atrio-ventricular canal was associated with an auricular septal defect. Polymicrogyria and pachygyria were seen at MRI examination. Cytogenetic investigation from blood and skin samples demonstrated mosaicism for a dicentric chromosome 11, as seen with G- and R-banding. C-banding demonstrated the presence of 2 centromeres, with a small interposed euchromatic extra-band. Parental karyotypes were normal. Using a whole chromosome painting probe, we demonstrated an intra-chromosomal rearrangement. With YAC probes, we were able to further characterize this rearrangement as a duplication of the proximal part of the long arm of chromosome 11q (band q12-q13). To our knowledge, this is the first report on trisomy of this part of the genome resulting in an abnormal phenotype.

Program Nr: 879 from the 1999 ASHG Annual Meeting

Isolated Growth Hormone Deficiency Associated with de Novo Balanced 46,XX, t(3;20)(q24;p13). *C. Duran[2], R. Romero[1], JC. Prieto[1,2], P. Sanchez[1], OL. Gutierrez[1], L. Jimenez[3].* 1) Departamento de Genetica Clinica Hospital la Victoria. Secretaria Distrital de Salud Bogota, Colombia; 2) Instituto de Genetica Humana. Universidad Javeriana, Bogota, Colombia; 3) Servicio de Endocrinologia Hospital San Ignacio. Bogota, Colombia.

The growth hormone deficiency is a cause of proportionate short stature, and represents a heterogeneous group of disorders secondary to a variety of genetic and acquired defects in human growth hormone (hGH) secretion or action. The frequency of hGH deficiency is estimated to range from 1:4000 to 1:10000. The hGH deficiency has been described with 18p- and 20p- chromosomal deletions, and rarely associated with 47XXY, 49XXXXY, and ring 5. We report on 10 year-old girl with proportionate short stature, high-pitched, retardation of growth and skeletal maturation; skeletal radiographs, thyroid hormone/thyroid-stimulating hormone were normal, basal Serum hGH levels <10 ng/ml, and the provocative tests of hGH secretory with clonidine suggests hGH deficiency. Chromosomal analysis performed using high-resolution R banding showed that the patient had a balanced translocation between the long arm of chromosome 3 and the short arm of chromosome 20. The parents were chromosomally normal. Thus, the karyotype of the patient was interpreted as 46,XX,t(3;20)(q24;p13), de Novo. The break at 3q24 or at 20p13 may have disrupted a gene responsible for the growth hormone deficiency locus. In a screen of the human genome for hGH and GNRH genes. One of these, designated Gonadotropin-releasing hormone 2(GNRH2) has been mapped to 20p13 and expression of GNRH2 suggests it may have multiple functions. In the band 3q25-q26.1 has been confirmed the localization of Short Stature Homeo Box 2 (SHOX2). The SHOX genes are implicated in craniofacial, brain, heart, and limb development. The new case further supports the assignment of the gene(s) responsible for hGH deficiency to 3q24 or 20p13. To our knowledge there are no similar reports involving translocations in hGH deficiency. A possible assignment of a new locus is discussed.

Program Nr: 880 from the 1999 ASHG Annual Meeting

Phenotypic effects of a 7p13p21 interchromosomal duplication including GLI3, TWIST and HOXA genes. *H. ELGHEZAL[1], A. MEGARBANE[2], P. GOSSET[1], G. JOLY[1], M. LE LORC'H[1], M. PRIEUR[1], M. VEKEMANS[1], C. TURLEAU[1], S.P. ROMANA[1]*. 1) Department of Genetics, Hopital Necker-Enfants Malades, Paris, FRANCE; 2) Department of Genetics, Saint Joseph University, Beyrouth, LEBANON.

Here we report on the phenotype of a man with a de novo insertion into 8p. HRB did not allow us to identify the chromosome material of this insertion. Spectral karyotype and CGH were performed and showed an 7p13-7p21 insertion in 8p23.1. FISH with probes corresponding to GLI3, TWIST and HOXA genes demonstrated that these genes were included in the rearrangement. The GLI3 (7p13), the HOX A (7p15) cluster and the TWIST (7p21) genes are of particular importance for their implications in dysmorphic syndromes. Haploinsufficiency of GLI3 and TWIST are responsible for two dominantly inherited malformative diseases, respectively the Greig cephalopolysyndactyly and the Saethre-Chotzen acrocephalosyndactyly syndromes. However the phenotype associated with triple dose of these two genes is not well documented. The HOXA cluster, contains 11 genes and, as the 3 others HOX genes clusters, is involved mainly in the determination of the body axis and in the limbs patterning. In human, except for the sympolydactyly and the hand-foot-genital syndrome due respectively to HOXA13 and HOX D13 dominant negative mutations, no homeotic transformation has been reported. Also the human phenotype associated with triple dose of the HOXA cluster genes has not been well documented. A detailed analysis of our patient's phenotype showed that he was only moderately mentally retarded, mildly dysmorphic but did not present gross skull, limbs or vertebral abnormalities except for a kyphoscoliosis. Particularly no homeotic transformation was noted. Firstly, this observation illustrates the value of spectral karyotype, FISH and CGH experiments in allowing a precise definition of a complex chromosome rearrangement and refinement of phenotype-genotype correlations. Secondly, it shows that triple gene dosage for the GLI3 and TWIST genes is not associated with gross phenotypic features. Thirdly, the absence of homeotic transformation in this patient indicates that triple dose of the HOXA cluster has few phenotypic consequences.

Program Nr: 881 from the 1999 ASHG Annual Meeting

Mosaic tetrasomy 9p in a 12 year old boy with Hypomelanosis of Ito. *C. Espiritu[2], J. Leonard[1], A. Shanske[2].* 1) Coriel Institute for Medical Research, Camden, NJ; 2) Dept. of Pediatrics, Montefiore Medical Center, Albert Einstein College of Medicine.

Hypomelanosis of Ito(HI) is a heterogeneous disorder characterized by patterns of hypopigmented whorls, streaks and patches typically distributed along Blaschko's lines associated with CNS abnormalities.It is felt to result from somatic mosaicism for a gene defect lethal to ectodermal dreivatives.The majority of patients have been found to have a chromosome abnormality (J Invest Derm 103:141S-143S, 1994). About 40% of these have chromosome mosaicism usually involving the X chromosome.Tetrasomy 9p is associated with a specific phenotype with craniofacial abnormalities, congenital heart defects, genital abnormalities and moderate to severe mental retaardation in survivors.We have evaluated a 12 year old boy with mosaic 9p tetrasomy and HI.

GL was the product of a term uneventful pregnancy.His physical examination at 12 years revealed a stigmatized youngster whose height was 130 cm(<5%), weight 33 kg(<<5%), and HC was 51.5(<2%). He had microbrachycephaly with occipital flattening, downslanting palpebral fissures, a lop ear deformity, synophrys, a unilateral simian crease, bilateral cryptorchidism, and linear streaks and whorls of depigmented skin following Blaschko's lines.He was ambulatory and was able to communicate in 2 word phrases but required assistance with activities of daily living. Laboratory investigation revealed a normal male karyotype in cultured fibroblasts.However, tetrasomy 9p was observed in in 98% of lymphocytes: 46,XY{2}/47,XY,psu dic(9)(9pter>9q12::9q12>9pter).ish psu dic(9)(wcp9+){98}.

Chromosomal abnormalities have been reported in association with HI since 1985 (Neurology 35:607-610).Since then, many chromosomal abnormalities have been reported including ring chromosome mosaicism, mosaic tetraploidy and chimeras.There have been only 17 cases of tetrasomy 9p reported to date (Am J Med Genet 63:434-437 1996). Our patient has many of the phenotypic features of this disorder as well as pigmentary dysplasia. We feel that he represents the first case of HI caused by mosaic tetrasomy 9p.

Program Nr: 882 from the 1999 ASHG Annual Meeting

Prenatal Interphase FISH Using the AneuVysion Probe Set in over 10,000 samples. *L.L. Estabrooks, M. Sapeta, C. Lytle, P. Challinor, H. Dietrich-Cook, D. Crenshaw, P. Norvell, P. Shadoan, R. McKnight, N. Hunter, B. Ravnan, P. Mowery-Rushton, V. Suri, A.N. Lamb.* Genzyme Genetics, Inc, Santa Fe, NM.

We report on our experience with over 8500 prenatal interphase FISH cases (to be updated to over 10,000 cases by October) using the AneuVysion probe set which was FDA approved in the Fall of 1997. This probe set detects the centromeric regions of chromosomes 18, X, and Y and unique sequence regions at 13q14 and 21q22.2. To date, experience with these probes shows the following: False negative rate (<1%), false positive rate (0%), detection rate (99%), and false sex results (<1%). The uninformative rate (4%) and rehybridization rate (6%) are low. The most common reason for an uninformative result is the risk for maternal cell contamination in a bloody sample (48%). Our abnormality rate is 9%, but varies considerably when correlated with indication, from a low of 3% with AMA and abnormal serum screen to a high of 18% with abnormal ultrasound. The probe set detects approximately 80% of all clinically significant chromosome abnormalities found in this prenatal population. Although the informative and detection rates decrease slightly in samples from pregnancies greater than 30 weeks, this appears to be reflective of a lower rejection rate of suboptimal samples in this population. A few unique cases will be presented as well as other adjunct uses of prenatal interphase FISH that can be advantageous in prenatal diagnosis.

Program Nr: 883 from the 1999 ASHG Annual Meeting

MOLECULAR STUDIES ON A SMALL RING X CHROMOSOME IN A FEMALE WITH A SEVERE PHENOTYPE. *W. Fang[1], H.C. Lu[1], W.L. Chen[1], Y.M. Wang[1], W.T. Chaou[2], C.C. Hwang[1], L. Estabrooks[3], B.T. Wang[3].* 1) Medical Research, Changhua Christian Hospital, Changhua, Taiwan, ROC; 2) Pediatrics, Changhua Christian Hospital, Changhua, Taiwan, ROC; 3) Genzyme Genetics, Santa Fe, NM.

Mosaic 45,X/46,X,r(X) can be associated with Turner syndrome or a more severe phenotype including MR, growth retardation, cardiac defects and dysmorphic features. The difference is thought to reflect size of the ring, distribution of the cell lines, and whether or not a functional XIST (X chromosome inactivation specific transcript) gene is present on the r(X). A functional XIST gene is necessary for appropriate r(X) inactivation and often predicts a Turner syndrome phenotype where absence of the gene or presence of a dysfunctional XIST gene is associated with a more severe phenotype. We report molecular findings of a small r(X) in a baby girl who manifests a severe phenotype. Clinical features in the propositus include an atrial septal defect, growth retardation, dysmorphic facial features. The differential diagnosis included Kabuki syndrome. Chromosome analysis revealed mos46,X,r(X)[39]/45,X[11]. FISH studies confirmed that the ring originated from an X chromosome and appeared to have an XIST gene locus. To determine if the XIST gene on the r(X) is fully expressed, RT-PCR analysis was performed and the amplified products were subjected to automated sequencing testing for mutations in the XIST and androgen receptor (AR) transcripts. Both XIST and AR products were generated from this patient, supporting that the XIST gene is transcribing. Sequencing did not reveal any mutations. Determination of methylation status and thus expression of the XIST gene were attempted using the AR assay described by Allen et al. (1992). Our initial data suggest that XIST and AR genes are expressed; however, the AR assay did not support full inactivation of the r(X). We suggest that although transcription of an XIST gene is required for X inactivation, it may not be sufficient for successful X inactivation. There could be other factors affecting normal function of the XIST gene transcript acting in cis or trans. Alternatively, the non-random X inactivation could be uncovering an X-linked recessive disorder.

Program Nr: 884 from the 1999 ASHG Annual Meeting

Recombinant Down syndrome: A case report and literature review. *S.A. Farrell, S.J. Lazzaro, V. Zimmer, A. Clough, G. Chow, M.D. Speevak.* Dept Lab Medicine, Credit Valley Hosp, Mississauga, ON, Canada.

We report a case of a female identified at birth to have features of Down syndrome (DS) with an atypical karyotype. Chromosome analysis initially revealed a 46,XX,dup(21q).ish21(wcp21+). The father's chromosomes were normal. However, the mother was found to have mosaicism for a pericentric inversion of chromosome 21 (19/30 cells). The revised karyotype of the child was 46,XX,rec(21)dup(21q)inv(21)(p12q21.1)mat. We performed a literature review to aid in counselling the family about recurrence risks as well as the developmental outlook for this child. Ten comparable probands from four previous publications were identified. All cases of rec dup(21q) were secondary to a maternal pericentric inversion. Male carriers did not seem to be at risk of having offspring with the rec dup(21q), although the number of male carriers was limited. In those with rec dup(21q), the risk of congenital heart disease was similar to that of trisomy 21. As well, their facial appearance was suggestive of Down syndrome but perhaps less striking. Although the data are limited, there is an indication the developmental disabilities as well as the short stature are milder in these individuals than noted for trisomy 21. This observation suggests the region proximal to the duplication must contain genes contributing to the severity of these features in Down syndrome.

Program Nr: 885 from the 1999 ASHG Annual Meeting

Development of FISH for Human Heart Tissue. *O. Faye-Petersen[1], I.J. Barrett[2], D.K. Kalousek[2], K.D. Wenstrom[1].* 1) Depts. of Pathology, and Obstetrics and Gynecology, University of Alabama at Birmingham, Birmingham, AL; 2) Dept. of Pathology, University of British Columbia, Vancouver, BC, Canada.

Congenital cardiovascular malformations (CCVM) affect approximately 8/1000 live born infants annually, and many have high rates of associated morbidity and mortality. However, despite their frequency, and rates of complication and death, the precise causes and/or mechanisms involved in their pathogeneses remain largely unknown. To date, archived samples from congenitally diseased hearts, submitted for diagnostic purposes at the time of autopsy or surgical palliative or corrective procedures, have been unavailable for research, because of the lack of methodology for cytogenetic analysis of formaldehyde fixed, paraffin embedded myocardium. Moreover, studies which have employed fresh tissue have been largely limited to the use of animal model myocardium. We report a novel method successfully permitting fluorescence in situ hybridization (FISH) to be used to detect genomic abnormalities in human, nondividing myocardial cells. Method: Parallel samples of fresh myocardium from routine autopsy were either frozen, formalin fixed, or formalin-fixed, paraffin embedded. Frozen samples were pepsin HCl digested to yield a single cell suspension, then hybridized with a DIG labeled centromeric probe for Chromosome 2 or 7 and the Di George/VCFS region probe. Formalin fixed material was sequentially digested with pepsin HCl and trypsin EDTA. Paraffin sections (4) were treated with sodium bisulfite and pepsin HCl prior to hybridization with centomeric probe for 16 and Di George probe. Findings: Discrete signals were obtained from all cell preparations. Conclusions: FISH analysis can now be utilized to study the potential roles of recently identified genomic aberrations in the occurrence of syndromic and nonsyndromic CCVM. Fresh frozen tissue is preferable for FISH analysis. Cytogenetic study of CCVM will be less restricted to kindred analysis; institutional surgical and autopsy pathology reservoirs of stored heart tissues can now be accessed and/or prospectively built for investigations of common and uncommon forms of CCVM.

Program Nr: 886 from the 1999 ASHG Annual Meeting

Unbalanced familial cryptic translocation t(8;11) in two fetuses with Beckwith-Wiedemann features. *S. FERT-FERRER[1], A. GUICHET[2], J. TANTAU[1], A.L. DELEZOIDE[1], C. OZILOU[1], S.P. ROMANA[1], P. GOSSET[1], G. VIOT[1], S. LOISON[1], C. MORAINE[2], N. MORICHON-DELVALLEZ[1], C. TURLEAU[1], M. VEKEMANS[1], M. PRIEUR[1].* 1) Department of Genetics, Hopital Necker-Enfants Malades, PARIS, FRANCE; 2) Genetics Unit, CHR Bretonneau, TOURS, FRANCE.

We describe a cryptic translocation ascertained after two induced abortions in the same family. Both fetuses had similar abnormal findings on ultrasound examination. Previous cytogenetic studies performed on cultured amniotic fluid cells were considered as normal in both fetuses. However, high resolution banding analysis and FISH studies performed on the parents revealed a paternal translocation t(8;11)(p23.2;p15.5). Retrospective FISH analysis on both fetuses showed that they carried a derivative chromosome 8, resulting in a partial deletion of 8pter and a partial trisomy of 11pter. Also a detailed examination of the fetuses phenotype revealed some features suggestive of BWS. Indeed FISH analysis using a IGF2 probe demonstrated the presence of three copies of the IGF2 gene. This study highlights the importance of cryptic subtelomeric rearrangements in pregnancies with recurrent unexplained multiple malformations syndromes discovered prenatally and contributes to a better delineation of the prenatal phenotype of BWS.

Program Nr: 887 from the 1999 ASHG Annual Meeting

First report of an unstable marker chromosome in a child with attention deficit disorder. *D.B. Flannery[1], H.B. Radtke[2], K.N. Norris[1], A.S. Kulharya[1]*. 1) Dept Pediatrics, Sect Genetics, Medical Col Georgia, Augusta, GA; 2) Dept Genetics, Children's Hospital of Wisconsin, Milwaukee, WI.

We present a first report of the observation of an unstable marker chromosome. While occurring with a variable incidence in different tissues of an individual, marker chromosomes are usually stable in size and morphology. Exceptions are seen in neoplasms. Review of the literature failed to identify any previous reports of an unstable marker chromosome.

This 10 year old boy was referred for Fragile-X testing because of speech delay, learning disabilities, sensitivity to noise, and attention deficit disorder. His prenatal and perinatal histories were benign. Physical examination was normal, except for numerous nevi. EEG and CT were normal. Peripheral blood chromosome analysis demonstrated mosaicism for a marker chromosome in 2 of 50 cells. The marker was not found in fibroblasts. Parental karyotypes were normal. On a repeat peripheral blood chromosome analysis the marker was seen in 6 of 150 cells. The size and morphology of the marker was variable in each metaphase. Rod-shaped morphology of the larger marker indicated that it is not a ring. FISH with whole chromosome painting robes demonstrated that the marker chromosome was derived solely from chromosome 12. While the phenotype is not consistent with previously reported partial trisomy of 12, it seems likely that his developmental and behavioral problems are related to the chromosomal anomaly.

Program Nr: 888 from the 1999 ASHG Annual Meeting

Influence of CYP2E1 and GSTM1 polymorphism on the frequency of chromosome aberrations in lymphocytes of former alcoholics. *G.J.F. Gattas, M.C.T. Calabrez.* Dept. of Legal Medicine, Ethics and Occupational Health, Medical School, Sao Paulo University, Sao Paulo, SP, Brazil.

Epidemiological data have identified chronic alcohol intake as a significant risk factor for alimentary tract cancer including liver. Although the exact mechanisms are not known, experimental studies support that ethanol is a cocarcinogen and or a tumor promoter. Genetic polymorphism of xenobiotic enzymes as CYP (cytochrome P450) and GST (glutathione-S-transferase) have been implicated in inter-individual variations in susceptibility to cancer related to environmental exposure. Ethanol is in part metabolized by CYP2E1 that has been implicated as a mediator in alcohol-induced liver damage. GSTM1 detoxify a wide range of reactive metabolites produced from CYP-mediated activation. We investigated the frequency of structural chromosome aberrations (CA) in lymphocytes of 26 ex-alcoholics individuals with no tumors and 29 control subjects not screened for alcohol intake from Sao Paulo, Brazil (Gattas & Saldanha, 1997). A PCR-based method was used to determine polymorphism of the CYP2E1 (at the RsaI site) and GSTM1 enzymes and the amplified products were evaluated in 2% agarose gel. Cytogenetic analyses revealed a significant increase ($p < 0.001$) of the frequencies of cells with CA in the abstinent alcoholics (8.5%) compared with controls (3.5%). Association with CA and enzymes polymorphism was not observed in control subjects. Only among ex-alcoholics a statistically significant (Fisher's exact test / $p=0.04$) increase in baseline CA frequencies was detected in subjects with GSTM1 null genotype (30%). The same association was not observed ($p=0.9$) for CYP2E1 mutant genotype in alcoholics (11.5%). Despite the limited number of subjects genotyped, the results seem to indicate an association between CA and GSTM1 polymorphism in alcoholic individuals. (This work was partially supported by LIM-HC-FMUSP).

Program Nr: 889 from the 1999 ASHG Annual Meeting

Alternate centromere inactivation in a dicentric (Y;14) (q12;p11) associated with azoospermia. *M. Gentile, P. Losavio, F. Cariola, A.L. Buonadonna, E. Caroppo, G. D'Amato, A. Di Carlo.* Medical Genetics, IRCCS, Castellana (BA), Italy.

Balanced reciprocal Y/A translocations have been reported in association with male infertility. A correlation between Y breakpoint within the spermatogenesis AZF genes region and azoospermia and/or hypogonadism have been postulated. Such correlation could be either confirmed or negated by molecular studies which identify precise breakpoints involving Yq11 or Yq12. We had the opportunity to evaluate a 28-year-old azoospermic patient. All metaphases showed a translocation t(Y;14). A 14/22 (p14.1) centromere probe and a Y centromere (pZYA) both showed a signal on the translocation chromosome confirming it was dicentric. Every copy of the translocation chromosome had only one primary constriction; in most (90%) the Y centromere has been inactivated, but in some (10%) the 14 centromere has been inactivated. FISH with a YAC contig and PCR amplification of 27 Y specific sequences (STS) spanning the Y chromosome short arm and the euchromatic portion of the long arm allowed assignment of the breakpoint in the Yq12 region. The paternal karyotype was normal. In our patient FISH and molecular deletion mapping analysis allowed acute assignment of the Yq breakpoint at the junction of euchromatin and heterochromatin, distal to the AZF genes location (Yq11), supporting the hypothesis that in Y/A translocations the infertility might be explained by other factors. No clear correlations have been found between alternate centromere inactivation and phenotype. An epigenetic mechanism seems regulate centromeric activity. Peripheral blood and germ cells patients alphoid DNA deletions analysis could contribute to identify an association between azoospermia and translocation chromosome centromeric inactivation. Contributions from Project 126-5-98, Ministero della sanit.

Program Nr: 890 from the 1999 ASHG Annual Meeting

Endoreduplication in oral squamous cell carcinoma (OSCC). *B.M. Gharaibeh[1], X. Huang[1], W.S. Saunders[2,3], I. Petersen[4], S.M. Gollin[1,3]*. 1) Department of Human Genetics, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA; 2) Department of Biological Sciences, University of Pittsburgh; 3) University of Pittsburgh Cancer Institute, Pittsburgh, PA; 4) Institute of Pathology, University-Hospital Charité, Berlin, FRG.

Endoreduplication, a common form of polyploidization in a variety of tissues, is rarely reported in cancer [Li et al. (1996) Cancer Genet. Cytogenet. 87:7-10]. Chromosomes are thought to duplicate inside of the nucleus in an endomitotic division without spindle formation, resulting in the appearance of diplochromosomes. We and others have observed that OSCC are characterized by complex, near-triploid karyotypes with multiple numerical and structural abnormalities, including deletions, duplications, and translocations. In addition to these alterations, we observed metaphases comprised of diplochromosomes that most likely resulted from endoreduplication. Schmid demonstrated that diplochromosomes can lead to the formation of atypical metaphase plates composed of tri- or tetrapolar spindles [Exp. Cell Res. 42:201-204 (1965)]. Consistent with this interpretation, our study revealed multipolar spindles in our OSCC cell lines by immunostaining and abnormal mitoses in sections of paraffin-embedded tumors. We also saw defects in expression of key cytoskeletal proteins that may have contributed to the observed abnormal spindle structures. This study suggests that endoreduplication and multipolar spindles may lead to chromosomal instability in OSCC.

Program Nr: 891 from the 1999 ASHG Annual Meeting

DETECTION OF SOMATIC GENETIC CHANGES IN HUMAN ENDOMETRIOSIS DERIVED CELL LINE (FbEM-1) BY COMPARATIVE GENOMIC HYBRIDIZATION AND FISH ANALYSIS. *J. GOGUSEV[1], L. TELVI[2], M. DOUSSAU[1], S. DU MANOIR[3], M. LEVARDON[4], J. BOUQUET DE LA JOLINIERE[4]*. 1) INSERM U507, NECKER HOSPITAL, PARIS, FRANCE; 2) LABORATOIRE DE CYTOGENETIQUE, HOPITAL ST VINCENT DE PAUL, PARIS, FRANCE; 3) INSERM U184, IGBCM, ILLKIRCH, FRANCE; 4) SERVICE DE GYNECOLOGIE OBSTETRIQUE HOPITAL BEAUJON, CLICHY, FRANCE.

Endometriosis is a cause of infertility and pelvic pain in 10-15%; of women of reproductive age. The molecular basis of this condition includes defects in oncogenes (c-myc, c-fms, Ki-ras, e-erbB1), aromatases, metalloproteinases and growth factor expression, but is in large part unknown. Comparative genomic hybridization (CGH), FISH and conventional cytogenetic analysis were used to perform wide genome survey in recently established human endometriosis derived permanent cell line FbEM-1 (Hum.Reprod. Update, 3,117, 1997). Genomic DNA from FbEM-1 cells labelled with biotin-16-dUTP and normal genomic DNA digoxigenin-11-dUTP labeled, were hybridized to metaphase spreads from blood of a healthy donor. Labeled sequences were detected using avidin-FITC and antidigoxigenin-TRITC. Images from metaphase cells were captured and the ratios of the FITC/TRITC intensities calculated. The cytogenetic analysis showed a hypotriploid karyotype and multiple marker chromosomes including a der(1) complement with homogeneous staining region (HSR). A clonal aberration interpreted as der(5)t(5q34;6p11) was found in more than 70%;; of the nuclei. CGH demonstrated gain of chromosome 1,3,5,6,7 and 17, while chromosomes 9,11,12,13,18 and X were underrepresented. Distinct amplicons were detected on 1q, 5p, 6p and 17q. FISH with paints for chromosome 1 confirmed chromosome 1 trisomy. The translocation der(5) was confirmed by double-color FISH. According to the CGH profile showing DNA sequences increase at 17q, FISH with single locus probe confirmed amplification of HER-2/neu protooncogene in 16.2%; of the FbEM-1 nuclei. These results reveal that FbEM-1 is characterized by several molecular genetic alterations. Thus, FbEM-1 cells may be an useful model to investigate the role of tumor suppressor genes and/or oncogenes in development of endometriosis.

Program Nr: 892 from the 1999 ASHG Annual Meeting

Cryptic terminal rearrangements detected using 24-color karyotyping (M-FISH). *B.K. Goodman[1,2], V. Praphanphoj[1,2], G.H. Thomas[1,2], G. Stetten[1].* 1) Johns Hopkins University School of Medicine, Baltimore, MD; 2) The Kennedy Krieger Institute, Baltimore, MD.

Subtle abnormalities at chromosome ends are sometimes detected using high-resolution G-band analysis. Chromosome-specific subtelomeric probes now permit testing for the possibility of a very distal deletion when such an abnormality is suspected. The question remains, whether or not material from another chromosome is involved. We tested the ability of 24-color fluorescence in situ hybridization (M-FISH) to detect and identify subtle or cryptic rearrangements at chromosome ends. Four techniques were used for each suspected terminal rearrangement: 1) high-resolution banding (650 bands or greater), 2) individual sub-telomeric FISH probes, 3) multi-telomere FISH (Cytocell, Inc.), 4) SpectraVysion 24-color FISH (Vysis, Inc.). Thus far we have examined two cases of 22q13 deletion and a deletion (18)(q21.3) for the presence of translocated material. In both 22q deletions, M-FISH results were in agreement with the other FISH assays. One case was found to be an unbalanced terminal rearrangement [der(22)t(19;22)(q13.42;q13.31)mat]. The M-FISH analysis consistently detected the presence of chromosome 19 material on the end of the deleted 22. The second case appeared to be a simple terminal deletion by all methods. No evidence of a translocation was found. While the del(18q) was cytogenetically obvious, the identity of the terminal G-light band was in question. M-FISH analysis detected the presence of material from another chromosome at the very end of the deleted 18 in all but one very short metaphase spread. In 33% (5/15) of the cells, the Quips SpectraVysion software (Vysis, Inc.) suggested chromosome 21 as the source of the extra material. The material was inconsistently identified in the remaining 10 cells. Viewing separate color planes confirmed the presence on the der(18) of the chromosome 21-specific fluors, and FISH with a 21qtel probe confirmed the rearrangement. Our experience indicates concordance among high resolution G-banding, multi-telomere FISH, individual sub-telomeric probes and M-FISH in identification of the origin of small rearrangements at chromosome ends.

Program Nr: 893 from the 1999 ASHG Annual Meeting

Tetrasomy(13)(q32-qter) due to a neocentromere at 13q32 in a mentally retarded boy with progressive loss of function. *L.P. Govaerts[1], I.F.M. de Coo[2], M. van der Blij-Philipsen[3], C.H. Wouters[1], H.J.F.M.M. Eussen[1], Chih-yu Yu[4], D. Warburton[4], J.O. Van Hemel[1]*. 1) Dept. Clinical Genetics, University Hospital Dijkzigt, Rotterdam, Netherlands; 2) Dept of Child Neurology, Sophia Childrens Hospital, Rotterdam, The Netherlands; 3) Dept of Molecular Cell Biology and Genetics, P.O. Box 108, 5500 AC Veldhoven, The Netherlands; 4) Clinical Genetics and Development, Columbia University, New York, NY, USA.

Statement of purpose: We report on the clinical and cytogenetic findings in a developmentally retarded hypotonic boy with tetrasomy (13)(q32-qter) due to a neocentromere at 13q32. At the age of 5 he developed behavioral changes, epilepsy, progressive loss of function and hypertonia. He died at home at the age of ten. Cytogenetic methods and results: The standard karyotype (GTG banding) showed non-mosaic 47,XY,+M with euchromatine. Parental chromosomes were normal. Additional chromosome staining technics (CBG, NOR and DA/DAPI) were all negative. Centromere identification by FISH with pTRA.20 probe (# 15), L1.26 probe(# 13, 21), 237(# 14, 22) and r521 (ribosomal DNA) failed. Spectral karyotype analysis suggested the marker to be a der(13). With CGH a duplication of the 13qter region from q32 was obvious. Subsequent FISH with WCP #13 (Cambio) and TH13qter probe confirmed the idea of an invdup(13q). The double signals from the telomere probe TH13qter on both ends of the marker proved the #13 marker to be an invdup13q32 with a neocentromere formation. Further analysis on metaphase and interphase chromosomes with cosmid 2G4 localised at the ZIC2 gene (13q32) showed two very closely opposed signals on the invdup(13).

Program Nr: 894 from the 1999 ASHG Annual Meeting

Design of modified oligonucleotide probes to detect telomere repeat sequences in FISH assays. *J.G. Hacia[1], E.A. Novotny[1], R.A. Mayer[1], S.A. Woski[2], M.A. Ashlock[1], F.S. Collins[1]*. 1) GMMB, NHGI/NIH, Bethesda, MD; 2) Dept. of Chemistry, U of Alabama, Tuscaloosa, AL.

Much current interest surrounds the structure of mammalian telomeres, and consequently, a robust and inexpensive fluorescent in situ (FISH) assay for telomere detection would be of considerable utility in both the research and clinical area. FISH probes based on unmodified oligonucleotides of peptide nucleic acid (PNA) homologs, complementary to one strand or the other of the hexamer repeat have certain disadvantages that caused us to examine alternative substrates for telomere hybridization. A series of dye-labeled oligonucleotide probes containing base and sugar modifications were tested for the ability to detect telomeric repeat sequences in FISH assays. These modified oligonucleotides, all 18-nt in length, were complementary to either the cytidine-rich $(C_3TA_2)_n$ or guanosine-rich $(T_2AG_3)_n$ telomere target sequences. Oligonucleotides were modified to either increase target affinity by enhancing duplex stability (2'-OMe ribose sugars and 5-(1-propynyl)-pyrimidine residues) or inhibit the formation of inter- or intramolecular structures (7-deazaguanosine and 6-thioguanosine residues) which might interfere with binding to target. Several dye-labeled oligonucleotide probes were found that could effectively stain the telomeric repeat sequences of either cytidine- or guanosine-rich strands in a specific manner. Such probes could be used as an attractive alternative to PNAs for investigating the dynamics of telomere length and maintenance. In principle, these relatively inexpensive and readily synthesized modified oligonucleotides could be used for other FISH-related assays such as triplet repeat expansions.

Program Nr: 895 from the 1999 ASHG Annual Meeting

Interstitial deletions of chromosome 5 and 16 without phenotypic abnormalities: further confirmation. *J.L. Hand[2], V.V. Michels[2], R.P. Ketterling[1], M.J. Marinello[3], S.M. Jalal[1]*. 1) Cytogenetics Laboratory, Department of Laboratory Medicine and Pathology; 2) Department of Medical Genetics, Mayo Clinic, Rochester, MN; 3) Genetic Diagnostic Laboratories, Inc., Buffalo, NY.

We describe two cases of euchromatic interstitial deletions without detectable phenotypic abnormalities. Patient one was a 19-year-old male who presented with progressive encephalopathy, progressive blindness, deafness, and spastic quadriparesis and was subsequently diagnosed with a peroxisomal disorder. Despite extensive evaluation, the specific peroxisomal disorder was not identified. We hypothesize that the son's phenotype may represent unmasking of a new autosomal recessive peroxisomal disorder in the deleted region. High-resolution chromosome analysis was interpreted as 46,XY,del(5)(p14.1p14.3). The asymptomatic mother had the same interstitial deletion. Use of wcp5 and SKY analysis confirmed that the abnormal chromosome 5 was not involved in a translocation. Chromosome 5p14 deletion has been reported previously in a family for three generations without phenotypic anomaly that included female to male and female to female transmissions. In the second case, an amniocentesis for advanced maternal age revealed an interstitial deletion in the long arm of chromosome 16. The karyotype of the fetus was 46,XX,del(16)(q13q22). The wcp16 painted the abnormal and the normal 16 entirely and there was no evidence of the presence of chromosome 16 DNA on any other chromosome. The pregnancy was carried to term and resulted in the birth of a normal daughter. The same deletion was observed in the phenotypically normal mother and a normal first child who is a 2-year-old son. Interstitial deletion of 16q21 has been reported previously to have a normal phenotype in a two generational family. These deletions do not seem to be associated with an abnormal phenotype due to imprinting. Interstitial euchromatic deletions without an apparent phenotypic anomaly include: Xq26,5p14,11p12, 13q21 and 16q21, a list that is growing.

Program Nr: 896 from the 1999 ASHG Annual Meeting

Y-Autosome Translocations characterized by classical banding techniques and molecular cytogenetic studies. *D. Hansmann[1], Z. Storm[1], R. Raff[2], G. Schwanitz[2].* 1) Institute Prenatal Diagnosis, Meckenheim, Germany; 2) Institute of Human Genetics, University of Bonn, Bonn, Germany.

Y-Autosome translocations are rare events of chromosome rearrangements. In our own investigation group of about 30000 chromosome analyses from lymphocyte culture we diagnosed ten cases. 5 of them had breakpoints in euchromatic regions and showed a phenotype in agreement with the unbalanced karyotype. The other 5 cases were rearrangements with chromosome 15 and breakpoints in the constitutive heterochromatin. In chromosome 15 the breakpoints were located between 15p11.2 and p12/13, in the Y-chromosome between Yq11.23 and Yq12. In these 5 cases, where we investigated the probands and their relatives, each person was carrying only one of the translocation chromosomes, this means that the rearrangement had occurred in earlier generations. The translocations could be delineated by a combination of different banding techniques and FiSH. The way of genetic counselling is given, 2 of the pedigrees are demonstrated in detail.

Program Nr: 897 from the 1999 ASHG Annual Meeting

Generation of repetitive sequence-depleted microdissected chromosome arm painting probe. *H. He[1], W. Huang[1], XY. Guan[2]*. 1) American Lab Technologies, Inc, Rockville, MD; 2) Department of Clinical Oncology, University of Hong Kong, Hong Kong, China.

The application of fluorescence in situ hybridization (FISH) with whole chromosome painting probes, chromosome arm painting probes, and chromosome band-specific painting probes has greatly facilitated to detect chromosome rearrangements in both hereditary diseases and cancers. To meet the increasing demand for the high quality FISH painting probes, a subtraction strategy has been applied in our company to remove repetitive sequences from microdissected DNA probes before hybridization. Chromosome arm 5p has been chosen to test our new method. Briefly, 10 copies of 5p were microdissected and amplified using a degenerate oligo primer (UN1) by PCR. UN1 primer was then replaced by a unique sequence primer (R1) by PCR. These PCR products were then hybridized with biotin-labeled human repetitive sequences derived from a 120 kb BAC containing various human repetitive sequences. After hybridization, avidin was added into the reaction and phenol/chloroform subtraction was performed to remove proteins in the reaction solution including all avidin-bound biotin-labeled repetitive sequences and their specifically hybridized repetitive sequences in the microdissected DNA. The remaining unique DNA fragments were recovered by PCR with R1 primer. The intensity and specificity of the repeat-depleted 5p arm painting probe have been characterized by FISH without adding block DNA Cot-1. The intensity and hybridization specificity of the fluorescence signal was similar between a regular 5p arm painting probe with Cot-1 block and our repeat-deplete 5p painting probe. This repeat-depleted painting probe which no longer require adding block DNA will be used for FISH and will provide cheaper and quicker resource for the increasing demand of the high quality FISH painting probes.

Program Nr: 898 from the 1999 ASHG Annual Meeting

Comparison of secondary cytogenetic abnormalities in pediatric T-cell versus B-cell lymphomas. *C.M. Higgins, M.M. Hess, B. Gordon, W.G. Sanger.* Human Genetics Laboratory, Univ Nebraska Med Ctr, Omaha, NE.

Cytogenetics of non-Hodgkin's lymphoma (NHL) have not been as intensively studied in children compared to adults. In childhood NHL, the majority of cases are neoplasms of B-lymphocyte origin (70%). Fewer lymphomas are of T-cell origin and most of these fall into a group of large cell lymphomas (LCL) known as anaplastic large cell lymphomas (ALCL). We present the cytogenetic and histologic results of eight t(2;5)-positive T-cell ALCL and compare the secondary clonal abnormalities with those of eleven pediatric B-cell neoplasms. Recurrent chromosome abnormalities in ALCL include t(2;5)(p23;q35) which leads to fusion of the nucleophosmin (NPM) gene on 5q to the anaplastic protein kinase (ALK) gene on 2p. This and other cryptic rearrangements may be important in the deregulation of ALK causing the pathogenesis of ALCL. The most frequent secondary cytogenetic features of ALCL from this analysis include the following disruptions, listed in decreasing frequency: 1q21, 10q24, 1q10, 3p23, 11q13, and 10q22. Loss of chromosome 4 and gain of the X chromosome were the most frequent numeric aberrations. Numerous breakpoints were noted on the long arms of chromosomes 1, 10, 11, and the entire length of chromosome 17. Most karyotypes were hyperdiploid followed by near-tetraploidy. Secondary to disruptions of 14q32 and 8q24, the B-cell lymphomas had a much more diverse pattern of additional abnormalities than the ALCLs. In decreasing frequency, repeated disruptions were at band 1q21, 1q42, and 15q26. The loss of Y and chromosomes 3, 4, and 15, in addition to extra copies of 8 and 18 were recurrent numeric abnormalities. Only chromosome 1 revealed a pattern of breakpoints along its entire length. Most of the pediatric B-cell cases were diploid and hyperdiploid. Similarities between the two groups of lymphomas were restricted to breakpoints clustered at 1q21 and the repeated loss of chromosome 4. The ALCL group had a much more consistent pattern of secondary abnormalities than did the B-cell tumors. Continuing studies may help elucidate the pathogenesis and progression of these pediatric lymphomas. Supported in part by the National Childhood Cancer Foundation.

Program Nr: 899 from the 1999 ASHG Annual Meeting

Aneuploidy for chromosomes 12, 13, 17, 18, X and Y and deletions on the Y chromosome in sperm from twenty oligoasthenoteratozoospermic (OAT) patients undergoing ICSI. *S.F. Hoegerman[1], M.G. Pang[2], M.K. Rudd[1], N.K. Dahiya[1], M. Stacey[1], L. Lunsford[1], G. Doncel[3], A.A. Acosta[3], W.G. Kearns[1,3,4].* 1) Cent for Ped Res, EVMS, Norfolk, VA; 2) Biomedical Res Cent, Korea Adv Inst of Science and Tech, Taejon, Korea; 3) Jones Inst, East VA Med Sch, Norfolk, VA; 4) Inst of Genet Med, Johns Hopkins Univ School of Med, Baltimore, MD.

Objective: To determine aneuploidy frequencies and Y chromosome deletions from twenty OAT patients and six proven fertile donors. **Study Design:**Multi-probe, multi-color fluorescence *in situ* hybridization (FISH) and STS-PCR was performed on sperm to determine aneuploidy and Y chromosome deletions. **Materials and Methods:**Aneuploidy frequencies were determined in sperm from twenty OAT patients and six controls. Three-probe, three-color FISH was performed using direct labeled DNA specific for chrom. 12,13 and 17 (probe set I) and chrom. 18,X and Y (probe set II). Over 104,000 sperm were scored in this study. STS-PCR was performed to determine genomic deletions within the Y chrom. **Results:** In OAT patients, the per chrom. disomy for chrom. 12 ranged from 0.3 to 4.3%, for chrom. 13 from 0.2 to 3.5%, and per chrom. disomy for chrom. 17 ranged between 0.09 and 2.4%. In controls, the mean per chrom. disomy frequency was 0.8% for chrom. 12, 0.2% for chrom. 13, and 0.2% for chrom. 17. The per chrom. disomy for the sex chrom. ranged between 1.8 and 5.3% and the per chrom. disomy for chrom. 18 ranged between 0 and 1.5%. In controls, the mean disomy frequency for the sex chrom. was 0.4% and the mean disomy frequency for chrom. 18 was 0.2%. Diploidy ranged between 0.1 and 2.6% in OAT patients, with a control mean of 0.3%. Total aneuploidy ranged from 31 to 70% for OAT patients. Total aneuploidy in controls ranged between 4.1 and 7%. STS-PCR studies from 20 OAT patients identified one Y chromosome deletion, from both sperm and somatic cells of one patient. From this same patient, a significant increase ($p<0.05$) in autosomal and sex chromosome aneuploidy was found in his gametes. **Conclusions:**These findings showed significant increases of genetic abnormalities in sperm of all OAT patients studied.

Program Nr: 900 from the 1999 ASHG Annual Meeting

Identification of a human autoimmune serum containing antibodies against the inactive X-chromosome. *B. Hong[1], M.S. Swanson[2,3], T.P. Yang[1,3,4].* 1) Department of Biochemistry and Molecular Biology; 2) Department of Molecular Genetics and Microbiology; 3) Center for Mammalian Genetics; 4) Division of Pediatric Genetics, University of Florida College of Medicine, Gainesville, FL.

Dosage compensation of X-linked genes in female mammals is achieved by transcriptional silencing of one of the two X-chromosomes. Structurally, the inactive X chromosome appears as a distinct heterochromatic nuclear inclusion, or Barr body. XIST RNA, a large non-coding nuclear RNA, is associated with the Barr body and is essential for X inactivation. However, it has been shown that XIST RNA is not directly bound to the X chromosomal DNA, suggesting that protein components of the Barr body may bind XIST RNA to the DNA by forming protein-RNA complexes. So far, such protein components have not been identified. Autoantibodies have been used as research tools for elucidation of intracellular structure and function. To identify antibodies that recognize components of the Barr body, we screened a total of 255 human autoimmune sera by indirect immunofluorescence microscopy. A variety of staining patterns by these autoimmune sera was observed. One serum sample recognized a Barr body-like heterochromatic structure. Further immunofluorescence studies with cultured human fibroblasts carrying different numbers of X-chromosomes demonstrated that the number of Barr body-like structures stained by this serum corresponded to that expected from the "n-1" rule of X inactivation. These results indicate that we have identified an autoimmune serum that contains antibodies against one or more molecular components of the Barr body. Identification of the antigen(s) is currently under way.

Program Nr: 901 from the 1999 ASHG Annual Meeting

The Neocentromere and Centromere Protein A (CENP-A). *E.V Howman, P. Kalitsis, K. Fowler, A. Newson, S. Redward, A. MacDonald, K.H.A Choo.* Chromosome Research, The Murdoch Institute, Royal Children's Hospital, Melbourne, VIC, Australia.

We previously identified a marker chromosome, mar del (10), in a patient who presented with mild developmental delay. A latent centromere was activated at the 10q25 region. This neocentromere lacks alpha-satellite and CENP-B. Using positional cloning, an 80kb region spanning this neocentromere was obtained and subsequently sequenced. A comparison between this region and the corresponding region of the normal 10 proved unremarkable. The activation of the neocentromere is therefore due to an epigenetic mechanism that has yet to be elucidated. An ideal candidate for this epigenetic role is CENP-A. This 17kD protein is restricted to active centromeres and its expression is synchronous with the late replication of centromeric DNA. CENP-A is a histone H3-like protein and a nucleosomal component. To determine the biological function of Cenpa, we generated mouse mutants by homologous recombination. Exon 2 of the Cenpa gene (amino acids 29-64) was deleted. This corresponds to the N-helix domain and a partial deletion of the helix 1 domain which are essential for centromere targeting. Heterozygous mice were produced and appear healthy. Current studies are focused on the production of null mice and the elucidation of the corresponding phenotype.

Program Nr: 902 from the 1999 ASHG Annual Meeting

Aneuploidy, chromosomal instability, and cytoskeletal defects in oral squamous cell carcinoma (OSCC) cells. *X. Huang[1], W.S. Saunders[2,3], B. Gharaibeh[1], M. Shuster[2], A.H. Enyenihi[2], S.M. Gollin[1,3].* 1) Department of Human Genetics, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA; 2) Department of Biological Sciences, University of Pittsburgh; 3) University of Pittsburgh Cancer Institute, Pittsburgh, PA.

OSCC are characterized by complex, often near-triploid karyotypes with structural and numerical variations superimposed on the initial clonal chromosomal alterations. The most likely reasons for the chromosomal instability in the OSCC cells are: 1) defects in the mitotic machinery of the cell, preventing normal mitotic chromosome segregation; 2) loss of function of the gatekeeper genes in the cell, preventing detection of DNA damage and cell cycle arrest; and 3) defects in cell cycle regulation, preventing the recognition and correction of segregational errors. We used immunohistochemistry and spectral karyotyping to study the chromosomal segregation and cytoskeletal defects and the relationship between them in cultured OSCC cells. In metaphase and anaphase cells, multipolar spindles and lagging chromosomes are detected frequently, suggesting the presence of defects in the mitotic apparatus and resulting chromosomal instability. Dicentric anaphase chromatin bridges and gene amplification suggest the occurrence of breakage-fusion-bridge (BFB) cycles. Some anaphase bridges persist into telophase, resulting in aberrant chromosomes that are excluded from the reforming nucleus, presumably leading to the micronuclei often observed in OSCC cells. Furthermore, half of the micronuclei are kinetochore-negative, suggesting chromosome breakage into acentric fragments. Our results suggest that cytoskeletal defects and BFB cycles may be responsible for some of the genetic instability in OSCC.

Program Nr: 903 from the 1999 ASHG Annual Meeting

Great Plains Cytogenetic Laboratory Trends between 1986-1996. *P.S. Ing[1], C. Johnson[2], S.R. Patil[2], W.G. Sanger[3].* 1) Boys Town Nat'l Research Hosp, Omaha, NE; 2) University of Iowa Hospitals and Clinics, Iowa City; 3) University of Nebraska Medical Center, Omaha.

Pooled data can be used as benchmarks for assessing laboratory quality assurance. Since 1986, the Great Plains Genetics Service Network Cytogenetics Committee has collected data on laboratory volume, failure rates, abnormality rates & turnaround times for different tissues: peripheral blood (PB), amniotic fluid (AF), chorionic villus (CVS), bone marrow (BM), tissue culture (TC), etc. from cytogenetic laboratories within the eight states of the region. In 1977, data items were modified. Not all labs provided all data on all items on all tissues in all years. As many as 20 different labs participated, from 9 labs in 1986 to 18 labs in 1991.

Laboratory volume across the region slowly increased from 12,000 samples total (14 labs) in 1987 to ~22,000 samples (16 labs) in 1996. PB & TC samples remained steady between 4,500 - 5,000 and 1,000 cases per year, respectively. AF samples rose steadily from >5,000 studies in 1987 to >8,000 in 1996. BM studies showed the greatest growth from ~1,400 studies in 1987 to ~6,000 in 1996. CVS studies peaked in 1991.

For **peripheral bloods:** failure rates dropped from 2.2% in 1986 to 1% in 1996; turnaround times were at 15 days in 1986, rose to 21 days in 1989, & dropped to 12.5 days for the last three years; abnormality rates remained fairly constant at ~15%.

For **bone marrows:** abnormality rates declined from 40% in 1986 to 22% in 1996 while failure rates remained steady at 8%; data were insufficient to calculate turnaround times.

For **amniotic fluids:** failure rates remained low (0.16% to 0.39%) while abnormality rates rose slightly from 2.5% in 1986 to 4% in 1996; data were insufficient to calculate turnaround times.

Supported in part by grant MCJ-191002, "Great Plains Genetic Service Network.".

Program Nr: 904 from the 1999 ASHG Annual Meeting

Cytogenetic Diagnosis of Fragile X Syndrome A study of 305 suspected cases in Saudi Arabia. *M.A. Iqbal, N. Sakati, A. Nester, P. Ozand.* King Faisal Specialist Hosp&RC, Riyadh, Saudi Arabia.

Background: Fragile X syndrome is the most common cause of inherited mental retardation. Patients with fragile X syndrome show variable mental disability, typical long and narrow facial appearance with large ears and prominent fontanel and frequent macro-orchidism. It is generally associated with a fragile site at Xq 27.3, which can be observed in the metaphase chromosome following selective culture conditions. At the molecular level, the fragile X syndrome is associated with an amplification of CGG repeat sequence of the FMR1 gene. The prevalence estimates are reported as one per 1500 males and one per 2500 females. Estimated prevalence rates of fragile X syndrome in different ethnic groups range from 0.4 - 0.8 per 1000 in males and 0.2 - 0.6 per 1000 in females. In this study, we have determined the frequency of fragile X positive cases in 305 preselected patients. Materials and Methods: 305 Saudi patients with mental retardation/developmental delay/clinical suspicion of fragile X syndrome were screened for fragile X chromosome by cytogenetic methods. Results: The majority of patients (95.59%) screened were under the age of 20 years. Two-hundred and ninety nine patients (98.03%) were in the category of mild to moderate mental retardation group. The frequency of fragile X positive cells in males ranged between 7% to 58% (mean 26 + 13.11), while in the females it was between 14% to 21% (mean 12.5 + 3.5) respectively. 24 males (7.86%) and two females (0.65%) were found to express fragile X site at q27.3. In this study 8.52% fragile X positive cases were found among 305 mentally retarded Saudi patients. Our findings are similar to the reports of frequency of fragile X cases observed in selected mentally retarded patients in other parts of the world. Molecular studies are currently in progress, and a correlation with cytogenetic and molecular fragile X analysis should give more insight into the etiology of this disorder.

Program Nr: 905 from the 1999 ASHG Annual Meeting

A high degree of mosaicism in preimplantation embryos from a carrier of a reciprocal translocation t(11;22)(q23;q11). *E. Iwarsson[1], H. Malmgren[1], J. Inzunza[2], L. Ährlund-Richter[2], B. Rosenlund[3], M. Fridström[3], P. Sjöblom[3], M. Nordenskjöld[1], E. Blennow[1]*. 1) Dept of Molecular Medicine, Karolinska Institutet, Stockholm, Sweden; 2) Department of Bioscience at Novum, Karolinska Institutet; 3) Department of Obstetrics and Gynaecology, Huddinge Hospital.

Introduction: Carriers of reciprocal translocations are at risk of having unbalanced offspring. Therefore, preimplantation genetic diagnosis (PGD) was performed on a woman carrying a reciprocal translocation t(11;22)(q23;q11). It is known that normal developing human preimplantation embryos show a high degree of aneuploidy/mosaicism. This prompted us to analyze all cells from 26 biopsied embryos in three treatment cycles for presence of unbalance and mosaicism. **Material and Methods:** Using three color fluorescence in situ hybridization (FISH) with two probes from chromosome 22 (22q11.2 and 22q13) in combination with a centromere-specific probe from chromosome 11, we examined the majority of cells from each embryo. **Results:** Three PGD treatment cycles were performed and one balanced embryo was transferred in cycle two and three. However, no pregnancy was established. In total, 26 biopsied, normal developing embryos were analyzed and a successful FISH analysis was achieved in 189 of 230 nuclei (82%). Twenty-three out of 26 embryos (88%) were mosaic and only 3 out of 26 embryos (12%) were balanced. For the two balanced embryos that were transferred, only the two biopsied cells were analyzed and a possibility of mosaicism remains. In the third balanced embryo there was no mosaicism. There were different degrees of mosaicism in the embryos. Some showed only two cell types (regarding the chromosomal content) while others displayed a different chromosomal content in every cell (chaotic chromosomal content). No embryo with homogeneously unbalanced chromosomal content was found.**Conclusion:** Compared to our own results and others, the number of abnormal embryos was higher (88%) than earlier studies (up to 75%) on normal developing preimplantation embryos from IVF patients without known chromosomal abnormality. These findings ought to be considered in the clinical preimplantation genetic diagnosis situation.

Program Nr: 906 from the 1999 ASHG Annual Meeting

Application of M-FISH for Identification of Markers and Derivative Chromosomes in Congenital Cases. *S.M. Jalal[1], M.E. Law[1], N.M. Lindor[2], G.S. Sekhon[3].* 1) Cytogenetics Lab, Mayo Clinic, Rochester, MN; 2) Department of Medical Genetics, Mayo Clinic, Rochester, MN; 3) Division of Clinical Genetics, University of Wisconsin, Madison, WI.

We have tested multicolor FISH (M-FISH), that became commercially available in 1998, in a series of normal and abnormal cases from blood, amniotic fluid, fibroblast culture, and bone marrow. We present the result of M-FISH analysis on a series of abnormal congenital cases. An extra ring was identified to be from chromosome 8 and confirmed by centromere-specific probe. In a family carrying a subtle t(4;8)(p16.1;p23.1) the der(4) and der(8) were detected in two members and confirmed by the chromosome-specific paint probes. However, the translocated segment of der(8) was detected by M-FISH with difficulty and had to be confirmed by 4p16.3 locus-specific probe. An add(4)(p16.1) was entirely from chromosome 4. Locus-specific probes for 4q16.3 and 4p12 were present once in this complex duplication. An add(7)(q26) was from 12 and confirmed by paint and telomere-specific probes. A suspected inv(18) was confirmed. An add(3) was entirely from 3. Telomere-specific probes indicated it to be a cryptic translocation involving dup(3qter) and del(3pter). A complex karyotype with an insertional translocation and balanced t(4;9) turned out to have the insertion but the translocation was complex t(8;10;12). M-FISH requires optimal hybridization for a distinct discrimination of classification colors, and the acrocentric fluorophores sometimes cross-hybridize to the acrocentric short arms. However, we find M-FISH to be an invaluable tool for resolution of markers, derivative chromosomes, and complex karyotypes.

Program Nr: 907 from the 1999 ASHG Annual Meeting

del(18p)SYNDROME WITH GH DEFICIENCY : REPORT OF 6 CASES AND REVIEW OF THE LITERATURE. *O. JARAMILLO LINES*[1], *C. SEVIN*[1], *L. TELVI*[2], *J.L. CHAUSSAIN*[1]. 1) ENDOCRINOLOGIE PEDIATRIQUE, HOPITAL ST VINCENT DE PAUL, PARIS, FRANCE; 2) LABORATOIRE DE CYTOGENETIQUE, HOPITAL ST VINCENT DE PAUL, PARIS, FRANCE.

More than 100 cases with 18p- have been reported until recently. Clinical features of these patients are variable. Patients presented caracteristic dysmorphia with mild to severe mental retardation. A short stature was found constantly. The etiology of the growth retardation is unknown. We described 6 patients with short stature (1,8SD to -6SD) and 18p monosomy (5 cases with del(18p) and 1 case with der18,t(13;18)(q11;p11.2)). Four patients had a partial and isolated GH deficiency, and 2 of them showed an abnormal pituitary gland after MRI examination. Two patients, out of 6, (one with GH deficiency and one with normal GH secretion) received GH treatment (0,8-0,9 IU/kg/wk) for 5 years. Height went up from -4SD to-2SD for the GH deficient patient and from -2SD to average for the normal GH patient. To date, 8 cases of 18p monosomy associated with short stature and GH deficiency was described. Three of them showed abnormalities of the development of pituitary gland. Four patients had GH treatment with a satisfactory growth rate. The 2 cases reported in this data, in addition to the 4 of the literature, showed that the patients with 18p monosomy and short stature should be considered for the GH treatment.

Program Nr: 908 from the 1999 ASHG Annual Meeting

Marker chromosomes are present at higher frequencies in older individuals with Down syndrome. *E.C. Jenkins, L. Ye, H. Gu, M. Genovese, C. Duncan, S. Brooks, N. Schupf, W.T. Brown.* NYS Inst Basic Res Dev Disab, Staten Island, NY.

Recently, we reported increased low-level chromosome 21 mosaicism in short-term whole blood cultures from older individuals with Down Syndrome (DS)(Am J Med Genet 1997;68:147-151). We observed a marker or acentric chromosome in 5% of the cells studied from a 45-year-old female subject. A number of deletions and age-associated losses of chromosomes 15, 17, 21 and X were also observed. We have now studied additional older individuals with DS and have observed marker chromosomes in 1 to 100% of cells examined from 5 older (40 years or older) individuals with DS, ranging in age from 43 to 61 years. Among a total of 324 individuals with DS studied, 5 or 1.5% have exhibited marker chromosomes. Among 148 individuals with DS who were under 40, none exhibited a marker chromosome, while 5 or 2.8% of 176 individuals with DS, 40 years or older, had marker chromosomes ($P<.05$, one-tailed), as compared to 1 or 0.4% marker chromosomes observed in 243 non-DS individuals with an otherwise normal karyotype ($P<.05$, one-tailed), who ranged in age from 40-88 years. It was possible to study two such marker chromosomes with M-FISH (multitarget fluorescence in situ hybridizaiton) followed up by whole chromosome painting via single DNA probe FISH analysis. One marker chromosome, from a 45-year-old female with DS, was present in 14 of 30 cells and was found to be derived from chromosome 6. The other marker, from a 54-year-old female with a 21q;21q translocation (trisomy 21), was a small metacentric chromosome that appeared to have been derived from chromosome 21 material, likely a result of Robertsonian fusion between two No. 21 chromosomes. This marker was present in 100% of 36 cells studied. To our knowledge, this is the first example of a mosaic marker chromosome 6 from an individual with DS. With this additional information, we now hypothesize that there is increased chromosome instability in cultures from older individuals with DS resulting in the formation of marker chromosomes, deletions, and selective, low-level non-disjunction. This work was supported in part by the NYS Office of Ment. Ret. and Devel. Disabil.; NIH: P01 HD35897-12, AG 14673; Alzheim. Assoc. RG3-96-077.

Program Nr: 909 from the 1999 ASHG Annual Meeting

A Ring Chromosome Derived From An Interstitial Deletion of The Short Arm of Chromosome 5. *M.T. Jodah[1], B.G. Kousseff[1], C.S. Kalter[2].* 1) USF Regional Genetics Program, Tampa, FL; 2) Florida Perinatal Associates, Tampa, FL.

Marker chromosomes are seen in 0.65-1.5 per 1,000 fetuses; 45% are familial. De novo markers have a risk of 10-15% risk for abnormalities. The size, staining properties and the presence of mosaicism are often useful in predicting their consequences. Ring chromosomes account for about 10% of all markers. On amniocytes, we encountered a de novo, ring chromosome derived from an interstitial deletion of the short arm of one chromosome 5. FISH analysis using an all-human centromere probe and a chromosome 5 painting probe confirmed there was an interstitial deletion of 5p. The ring represented the deleted segment of 5p with a centromere; the karyotype was 47, XY, del(5)(p13), +mar.de novo.ish (5)(p11p15.1). The genetic counseling for this patient was complicated. The initial concern was about cri-du-chat syndrome. Fortunately, FISH determination of the origin of the marker resulted in an improved prognosis. The patient was counseled regarding at least a 10% risk for phenotypic abnormalities and/or developmental delay. Clinical genetics evaluation is pending. Ring chromosomes are formed when there is a break in both arms of a chromosome with the resulting terminal segments lost and formation of a ring from the remaining segment or due to telomere-to-telomere fusion. Frequently they are unstable leading to varying levels of mosaicism. Interstitial deletions leading to ring chromosome are rare and have only been described involving chromosomes 2, 5, 9, 10, 16, and 17 (Petit & Fryns, 1997; Schuffenhauer, 1996). At least three mechanisms have been proposed to explain the occurrence of ring chromosomes.
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Program Nr: 910 from the 1999 ASHG Annual Meeting

SRY in XX males and XY females: Demonstration by primed in situ labeling. *J.S. Kadandale[1], Y. Tunca[1], R.S. Wilroy[1], P.R. Martens[1], S.S. Wachtel[2], A.T. Tharapel[1].* 1) Clinical and Molecular Cytogenetics Laboratory, Dept Pediatrics, Univ Tennessee, Memphis, TN; 2) Reproductive Genetics Laboratory, Dept Obstetrics & Gynecology, Univ Tennessee, Memphis, TN.

Primed in situ labeling (PRINS) is a sensitive and specific method that can be used to localize DNA segments too small to be detected by conventional FISH. We used the PRINS method to localize the SRY gene to the short arm of the Y-chromosome. We also demonstrated its presence on the Y chromosome in women with 46,XY gonadal dysgenesis, and identified an SRY domain on the der(X) in men with t(Xp;Yp). In addition, we performed PCR with conventional primers to amplify SRY in genomic DNA from the same subjects. The PRINS method that we employed involved significant modification of the existing methodology (which is used to identify repetitive sequences). Annealing of primers and extension were accomplished on chromosome preparations on microscope slides in the presence of labeled nucleotides and Taq DNA polymerase. SRY signals were detected at band Yp11.31p11.32 (ISCN 1995) in 95% of metaphase spreads. About 90-95% of interphase nuclei showed the SRY signal in the male controls. SRY signals were observed on the short arm of the der(X) from the 46,XX male and the male with 46,der(X),t(Xp;Yp),+mar, confirming Xp-Yp interchange. The signals were not detected in female controls. In view of our experience with SRY and five other genes, we conclude that PRINS is useful for routine localization of single copy genes and small DNA segments, in general.

Program Nr: 911 from the 1999 ASHG Annual Meeting

A rare terminal deletion involving the distal short arm of chromosome 12. *K.A. Kaiser-Rogers[1], K.W. Rao[1], M.I. Roche[1], C.M. Lese[2], C.M. Powell[1]*. 1) Dept Pediatrics, Univ North Carolina, Chapel Hill, NC; 2) Ctr Medical Genetics, Univ Chicago, Chicago, IL.

We are studying a 9 4/12 year old male with what appears to be the smallest cytogenetically detected terminal short arm deletion of chromosome 12 reported to date [46,XY,del(12)(p13.33)]. This deletion was so subtle that many of the examined cells looked normal by G-band analysis. A FISH study using a unique telomere probe for the short arm of chromosome 12 (BAC 124K20) was used to exclude the possibility that mosaicism was present in this patient. Additional FISH studies are currently in progress to further characterize this rearrangement.

J.D. presented as a small, thin child with a marfanoid habitus, mental retardation, behavior problems and involuntary movements involving his face, body and extremities. His height was 131.5cm (25th percentile), his weight was 20 kg (<5th percentile) and his head circumference was 47 cm (< 5th percentile). Several minor dysmorphic features were noted including small ears (3rd percentile), periorbital fullness, a narrow palate, and a small mandible. On the left hand, a single transverse palmer crease and an extra digital crease on the fourth finger were noted. Dermatoglyphics were also unusual with a predominance of whorls. His joints were mildly hyperextensible.

Comparison of our patient with those previously reported suggests that the most consistent clinical findings reported in terminal 12p deletion patients include growth retardation, microcephaly and micrognathia. Mental retardation was the only feature common to all patients. The involuntary movements observed in our patient have not been previously reported. At this time we do not know whether this dyskinesia is related to his medication(s) or is secondary to his deletion. Interestingly the gene for dentatorubral-pallidolysian atrophy (DRPLA), a progressive neurological disorder also known as Haw River syndrome, has been mapped to the distal long arm of chromosome 12.

Program Nr: 912 from the 1999 ASHG Annual Meeting

Double and Triple Aneuploidy In Products of Conception. *N.B. Kardon[1,2], T.M. Dunn[1], K. Hirschhorn[1,2]*. 1) Dept Human Genetics, Mount Sinai Medical Center, New York, NY; 2) Dept Pediatrics, Mount Sinai Medical Center, New York, NY.

Reports of double aneuploidy observed in products of conception as well as liveborns are infrequent. The occurrence of triple aneuploidy in cases of spontaneous abortions are extremely rare. From January 1998 through May 1999 our laboratory processed 320 products of conception (POC). Approximately 60% of these cases were diagnosed with an abnormality. In total there were 23 cases of complex aneuploidy. The 23 cases consisted of 17 cases of double aneuploidy; 2 cases of triple aneuploidy; one case each of five extra chromosomes and six extra chromosomes; trisomy mosaicism for two different chromosomes and a jumping translocation with aneuploidy. This represented 7% of the total POC specimens and 12% of the observed abnormalities. Only 4 cases had double aneuploidy involving sex chromosomes. None of the triple or greater aneuploid cases involved the sex chromosomes. The extra autosomes included various combinations of 2, 4, 5, 7, 8, 9, 10, 12, 13, 15, 16, 18, 19, 20, 21 and 22. The predominance of complex aneuploidy in POC specimens has not been previously reported. Most reports are of single cases without any indication of the frequency of the abnormality. While cases that survive to term include extra sex chromosomes, this the significance of various other combinations of chromosomes and recurrence risks have not been established. Our data identify a significant number of multiple aneuploidies in these specimens. It appears that multiple errors in a single conceptus are more common than is indicated in the literature. These errors appear to be random events with no significant prevalence. The consequences of not reporting complex aneuploidies due to the inability to correlate a specific phenotype with the SAB is that no true indication of frequency, follow up on future pregnancies or histories of current pregnancies are available. We will compare the frequencies of complex aneuploidy in the products of conception from our laboratory to those previously reported cases found in the literature.

Program Nr: 913 from the 1999 ASHG Annual Meeting

Double recombinant chromosome 22's in a family with an inherited pericentric inversion through 3 generations.
J.C. Kelly, S.M. Zneimer. Cytogenetics Laboratory, SmithKline Beecham Clinical Laboratories, Van Nuys, CA.

We report a rare family study of 3 generations with an inherited chromosome abnormality. A proband presented for amniocentesis due to abnormal ultrasound findings. Chromosome analysis of the amniocentesis showed a bisatellited chromosome 22. Fluorescence in situ hybridization (FISH) showed loss of the arylsulfatase (ARSA) locus (Vysis, Inc.) at 22q13.3 and duplication of the rRNA on the long and short arms of chromosome 22. Outcome of the pregnancy is pending. Parental blood chromosome analysis revealed a pericentric inversion of chromosome 22 in the father of the fetus. The father's karyotype was 46,XY,inv(22)(p11.2q13.3). The subsequent karyotype of the fetus was 46,XY,rec(22)dup(22p)inv(22)(p11.2q13.3)pat. Genetic counseling with the family revealed that the father of the fetus had a 21 year old brother with mental retardation. Subsequent chromosome analysis of the brother and their parents showed the brothers' 47 year old mother as a carrier for the inversion. The brother's chromosome analysis revealed the other type of recombinant chromosome 22. FISH studies confirmed the presence of separate, duplicated ARSA loci on the long and short arms of chromosome 22. His karyotype was 46,XY,rec(22)dup(22q)inv(22)(p11.2q13.3)mat. The phenotypic features of del(22)(q13.3) which was seen in the fetus with ultrasound abnormalities have been described in the literature. Major features include developmental delay, hypotonia, speech delay and mild facial dysmorphic features. Phenotypic features of the brother with the duplicated 22q13.3-ter bands include mild dysmorphic features and mental retardation. It is unusual to find both recombinant versions of an inherited pericentric inversion with viable offspring in a single family. Since chromosome 22 is small, one might expect both recombinant versions to be viable, however, the region of crossover during meiosis is small enough to give a low probability that both events would occur. Further clinical workup of the brother with mental retardation is underway as is a detailed pedigree analysis of relatives and their pregnancy histories.

Program Nr: 914 from the 1999 ASHG Annual Meeting

de novo Acute myelogenous leukemia(AML)-associated cytogenetic abnormalities in patients with acquired severe aplastic anemia. *S.H. Kim[1], K.A. Lee[1], H.R. Yoon[2]*. 1) Dept of Clinical Pathology, Samsung Medical Center, Sungkyunkwan Univ School of Medicine, Seoul, Korea; 2) Seoul Clinical Laboratories, Seoul, Korea.

There may be some of acquired severe aplastic anemia(SAA) that may have chromosomal abnormalities such as +8, -7 and 22q-, which are frequently associated with clonal disorders of multipotent stem cells, e.g. myelodysplastic syndrome(MDS) or secondary leukemia. These findings suggest that some SAA could be preleukemic clonal disorders, however, the clonal nature of SAA is still in debate. The cytogenetic studies could be helpful in the clonal study of SAA. Twenty-nine patients with acquired SAA underwent cytogenetic studies on bone marrow cells at the time of diagnosis. Of the 29 patients, 8 patients(27.6%) had chromosomal abnormalities at the time of diagnosis, which were trisomy 8 in two cases, t(8;21), inv(16), t(4;14), t(X;19), del(10) and monosomy 10, in one case each. Six patients underwent follow-up examination after immunosuppressive therapy(IS) and/or bone marrow transplantation. One patient with trisomy 8 showed persistent chromosomal abnormality after IS. Interestingly, two patients had t(8;21)(q22;q22) and inv(16)(p13.1q22), which are strongly associated with de novo AML(M2 and M4e), however these two patients did not evolve to MDS or acute leukemia after 16 and 28 months. The incidence of aplastic anemia appears to be relatively higher in Korea and the frequency of chromosomal abnormalities of SAA in this study seem to be higher than those of other previous studies. Our data suggest that a proportion of SAA might be associated with lineage commitment progenitor cell defect and has potentials for a myeloid specific leukemic evolution.

Program Nr: 915 from the 1999 ASHG Annual Meeting

De novo translocation between chromosomes 15 and 18 in prenatal diagnosis. *Y.M. Kim[1], S.Y. Park[1], M.Y. Kim[2], J.M. Kim[1], S.A. Nam[1], H.M. Ryu[2], I.S. Park[2]*. 1) Genetic Reserch Laboratory, Samsung Cheil Hospital, Seoul, Korea; 2) Obstetrics and Gynecology, Samsung Cheil Hospital, Sungkyunkwan University, School of Medicine, Seoul, Korea.

We report de novo t(15;18) in prenatal diagnosis. The patient was referred to us for nuchal fold thickness 5mm at 13.6wks of gestation. Amniocentesis revealed 45,XX,der(15)t(15;18)?(p11.2;p11.2),-18 by conventional GTG-banding. The level II sonography at 20wks didn't show any special physical abnormality other than right unilateral ventriculomegaly and nuchal fold thickeness 6.4mm without cystic hygroma. In the parental study, they showed normal karyotypes and had 1 healthy child and 3 spontaneous abortion, one due to 22 trisomy. To confirm the result of amniocentesis, the cordocentesis was performed at 21.4wks, and high resolution and RBG-banding showed same result as that of amniocentesis. Using comparative genomic hybridization(CGH) and local specific FISH we found out the partial monosomy of chromosome 18p.

Program Nr: 916 from the 1999 ASHG Annual Meeting

Interstitial deletion (1)(qter®p21::p22®pter) in a 22 month old boy. *E.P.E. Kirk[1], A. Daniel[2], Z. Wu[2], P.J. Grattan-Smith[3], M-Y. Yip[4].* 1) Dept of Medical Genetics, Sydney Children's Hospital, Randwick, NSW, Australia; 2) Department of Cytogenetics, Western Sydney Genetics Program, Westmead, NSW, Australia; 3) Department of Neurology, New Children's Hospital, Westmead, NSW, Australia; 4) Molecular and Cytogenetics Unit, South Eastern Area Laboratory Services, Randwick, NSW, Australia.

We report a 22 month old boy with an interstitial deletion of chromosome 1p. His clinical features include moderate global developmental delay, submucus cleft palate and craniofacial dysmorphism. He has marked brachycephaly, a flat facial profile with midface hypoplasia, small, cupped, lowset ears and a small, down-turned mouth. Cranial MRI at 14 months revealed mild global cerebral atrophy but was otherwise normal. The deletion was not detected on the initial study on blood lymphocytes. Subsequently, a karyotype was performed on cultured skin fibroblasts, because of the possibility of chromosomal mosaicism not detectable in lymphocytes. This was abnormal, with the karyotype 46,XY, del (1)(qter®p21::p22®pter). This finding was confirmed on review of the lymphocyte chromosomes. There have been eight previous patients with interstitial deletions of 1p reported, of whom two were identical twins. Both of the other reported interstitial deletions involving 1p21 were larger than that observed in our patient. The only clinical feature our patient has in common with these cases is developmental delay.

Program Nr: 917 from the 1999 ASHG Annual Meeting

Parental Origin and replication timing studies in a 70,XXXX liveborn. *J.H.M. Knoll[1,2], H. Baker[1], G. Cox[1], M.L. Begleiter[2], L.M. Pasztor[2].* 1) Division of Genetics, Children's Hospital, Harvard Medical School, Boston, MA; 2) Section of Medical Genetics and Molecular Medicine, Children's Mercy Hospital, Department of Pediatrics, University of Missouri School of Medicine, Kansas City, MO.

We describe the clinical, cytogenetic and molecular findings in a female with a 70,XXXX karyotype. Triploidy, while infrequent in liveborns, is even more rare when combined with X chromosome aneuploidy. E.G., a 32 3/7 week child born to a 27 y.o. G2P2 woman, exhibited severe growth retardation, facial dysmorphology, cardiac anomalies and Dandy Walker malformation. E.G. died on day 4 and an autopsy confirmed the physical findings and identified streak ovaries. The placental was small and immature. Cytogenetic analysis revealed 70,XXXX in all metaphases from blood (n=20) and four autopsy tissues. BrdU replication studies showed that three of the X chromosomes were late replicating. Consistent with the placental morphology, the extra haploid chromosome set was of maternal origin. Two distinct maternal alleles (heterodisomy) at D1S249, D15S817, D15S1043, DXS8043, DXS991 and one distinct maternal allele (isodisomy) at D4S402, D5S639, D13S170, DXS987, DXS1001 were observed. Methylation at SNRPN confirmed that the third chromosome 15 was maternally derived; however, the maternal chromosomes replicated sequentially rather than synchronously. It will be important to investigate whether the sequential replication observed in this case is characteristic of other tirploid and trisomic cell lines and whether it has a role in phenotypic outcome.

Program Nr: 918 from the 1999 ASHG Annual Meeting

Familial Mental Retardation Syndrome ATR-16 Due to an Inherited Cryptic Subtelomeric Translocation t(3;16)(q29;p13.3). *R.F. Kooy[1], E. Reyniers[1], S. Uhrig[2], I. Schoepen[1], A. Golla[2], J. Wauters[1], P. Kroisel[3], P. Bossuyt[1], I. Rost[2], K. Jedele[2], H. Zierler[3], S. Schwab[4], D. Wildenauer[4], M. Speicher[5], P.J. Willems[1], T. Meitinger[2], E. Holinkski-Feder[2].* 1) Dept Medical Genetics, Univ Antwerp, Antwerp, Belgium; 2) Department of Medical Genetics, University of Munich, Munich, Germany; 3) Department of Human Genetics, University of Graz, Graz, Austria; 4) Department of Human Genetics, University of Bonn, Bonn, Germany; 5) Department of Human Genetics, University of Munich, Munich, Germany.

The etiology of mental retardation can be established in less than 50% of all cases. Especially in the category of patients with mild to moderate mental retardation (IQ 50-70) the molecular cause is only determined in a minority of cases. These cases are rarely caused by metabolic disorders or teratogenic effects, and the combination of standard karyotyping and molecular analysis of the FMR1 gene leads to a diagnosis in less than 10% of the patients. Recently, chromosomal translocation and deletions that could not be detected by standard cytogenetic analysis have been identified in some of these patients. These cryptic (cytogenetically invisible) translocations of chromosome ends have been estimated to be implicated in 6% of cases of mental retardation that are otherwise karyotypically normal. Here we present a five-generation family including ten patients in generations IV and V affected with mild to moderate mental retardation and nonspecific dysmorphic features. Standard DNA and cytogenetic analysis was unable to determine the cause of the mental retardation. Only after linkage of the phenotype to chromosome 16p13.3 was FISH analysis able to detect an unbalanced subtelomeric translocation resulting in partial monosomy 16ptel and partial trisomy of 3qtel in all affected patients. Reevaluation of the dysmorphic features and red blood cell parameters indicated that the affected patients had clinical features compatible with ATR16 syndrome. Investigation with FISH then revealed a balanced translocation between telomeric 16p and 3q in obligate carriers. This case supports the role of subtelomeric chromosomal aberrations in familial mental retardation.

Program Nr: 919 from the 1999 ASHG Annual Meeting

Quantitative fluorescent PCR in the prenatal and postnatal detection of aneuploidies, loss of heterozygosity, interstitial deletions and of their parental origin. *A. Krebsova[1,3], I. Horka[1], S. Jobert[1], O.A. Haas[2], D. Chudoba[1], M. Koudova[1], J. Diblik[1], M. Macek Jr.[1], K. Sperling[3], M. Macek[1].* 1) Inst. Biol & Med. Genetics, 2.Sch. Med., Charles Univ. , Prague, Czech Rep; 2) CCRI, St. Anna Kinderspital, Vienna, Austria; 3) Inst. Hum. Genetics, Humboldt Univ., Berlin, Germany.

The aim of the study was to assess reliability of quantitative fluorescent PCR (QFPCR) in rapid detection of aneuploidies, interstitial deletions, loss of heterozygosity (LOH) and parental origin of chromosomes (13, 18, 21, X, Y) from micro-quantities of uncultivated cells. STR markers-D13S258, D13S631, MBP, D18S51, D18S535, D21S11, D21S1414, D21S1411, D21S1412, D21S167, XHPRT and AMX, AMY were examined on ABI 310 Prizm. QFPCR was tested in 369 cases on DNA from non-cultivated/cultivated amniocytes, non-cultivated placenta, mola hydatidosa, solid tumors and peripheral blood cells. The results of QFPCR were in 100% identical with prenatal cytogenetic diagnosis. Prenatal diagnosis was not possible in 8/200 cases, because QFPCR characteristic was inconclusive, according to our statistical analysis of normal peak height, size variability and diagnostically significant quantitative criteria for diallelic (2:1) trisomy. QFPCR was successful also in amniocytes from 0.1 ml of amniotic fluid and persisting in suspension. Parental origin of fetal aneuploidies was disclosed in 7 detected fetal aneuploidies and in 27/29 families with trisomy 21. The determination of meiotic origin of aneuploidy was possible in 21/27 cases. QFPCR of solid tumors and mola hydatidosa enabled rapid detection of aneuploidy and LOH, interstitial deletions invisible by the current cytogenetic methods. Analysis of control and tumor cells is necessary to discriminate monoallelic STR markers from LOH. Differentiation between total or partial monosomies, interstitial deletions, determination of their size requires more STR markes from centromeric-telomeric regions. QFPCR complements cytogenetic diagnosis, assures it even if it is impossible due to cultivation failure and contributes to the improvement oncogenetic studies. Supported by IGA 2899-5, 3526-3, 4124-3, OK 192.

Program Nr: 920 from the 1999 ASHG Annual Meeting

Duchenne muscular dystrophy in a girl with a balanced translocation [46,X,t(4;X)(q31.1;p21.1)]. *R.W. Kula[1,2], S.M. Kleyman[3], M.J. Macera[3,4], R.S. Verma[2,3,4].* 1) Long Island College Hospital, Brooklyn, NY; 2) SUNY Health Science Center at Brooklyn, NY; 3) Institute of Molecular Biology and Genetics at InterScience, Brooklyn, NY; 4) Wyckoff Heights Medical Center, Brooklyn, NY/New York Hospital-Weill Medical College of Cornell University, NY, NY.

An 11 year old African-American girl had a slight speech developmental delay and was noted to have large calves at age 21/2-3 years. At age 8, she was noted to have frequent falls. For the past few months, she has been using a wheelchair. On neurological examination, she walked with increased lordosis and had a tendency to toe walk. She has a positive Gower's maneuver and is unable to lift her head while lying flat. She has more prominent proximal, but significant gereralized weakness graded 4/5 in the upper extremities at worst and 3/5 in the lower estremities at worst. Her deep tendon reflexes were absent except 2+ at the ankles with flexor plantar responses. Clinical presentation was consistent with Duchenne muscular dystrophy. Cytogenetic findings with GTG- and FISH- techniques revealed an abnormal 46,X ish.t(4;X)(q31.1;p21.1)(wcpX+.wcp4+). Although the patient has an apparently balanced translocation, the breakpoint is located at Xp21.1 by high relolution banding technique. This observation suggests that a gene involved in muscular dystrophy must be at or around Xp21.1. Although the exchange points were not the same in earlier affected cases and the disruption region extends from Xp21.1 to Xp21.3, this finding clearly raises a sereious issue concerning inactivation, balanced translocations and obviously a critical region for this disease.

Program Nr: 921 from the 1999 ASHG Annual Meeting

Does anticipation exist for some familial chromosome abnormalities? *A.S. Kulharya, K.N. Norris, E.A. Sekul.*
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Unbalanced familial chromosome abnormalities have been occasionally reported in literature. Some of these abnormalities are without any significant phenotypic effect, while others show a more pronounced phenotype in the proband as compared to the carrier parent. It is unclear whether this is due to better awareness and availability of genetic services, or a phenomenon similar to anticipation exists for some of these unbalanced chromosome abnormalities. Wilson et.al. (1993)have suggested that for familial 22q deletions the phenotype is more severe in the child as compared to the carrier parent.

We report a familial interstitial duplication of 7(q32->36.1). The family history is significant for bipolar disorder. Mother has history of depression and manic behavior. She is mildly retarded and has difficulty in reading although she can write. The proband's maternal aunt and all four of her children are developmentally delayed. Great grandmother also had a nerve problem of unknown etiology. At 20 months the child did not walk or crawl. He did not have any dysmorphic features. The CT and EEG exams were normal. No organomegaly was noted. He has behavioral problems.

Chromosome analysis demonstrated interstitial duplication at the distal end of the long arm of a chromosome 7. Maternal blood chromosome analysis also showed the same abnormal chromosome 7. Father was unavailable for testing. FISH with a whole chromosome paint demonstrated that the extra material was derived from chromosome 7 itself. The karyotype of the proband is 46,XY,dup(7)(q32q36.1). Several cases have been reported with duplication of various regions of 7q. This patient does not have any dysmorphic features or skeletal abnormaities associated with similar duplications of 7q. However, there is a pronounced progression in the severity of developmental and neurological charecteristics as opposed to the carrier mother.

Program Nr: 922 from the 1999 ASHG Annual Meeting

Chromosome Abnormalities in a Consanguinous Couple with "Double Trouble". *G.S. Kupchik[1, 2], S.K. Barrett[1, 2], A. Babu[3], M.J. Macera[4], R.S. Verma[4].* 1) Dept Pediatrics, Maimonides Medical Center, Brooklyn, NY; 2) SUNY Health Sciences Center at Brooklyn, NY; 3) Beth Israel Medical Center, New York, NY; 4) Institute of Molecular Biology and Genetics at InterScience and Wyckoff Heights Medical Center, Brooklyn, NY.

In certain cultures, first cousin marriages are favored, while in others such unions are discouraged. We report a case of first cousin mating where both partners have the same balanced translocation involving chromosomes 16 and 18; i.e. t(16;18)(p13.2;p11.2). Consanguineous matings in such situations may produce progeny with balanced and unbalanced genotypes. By 29 years of age, our proband had two first trimester spontaneous abortions, a phenotypically normal daughter, and a son with multiple congenital anomalies and an unbalanced karyotype 46,XY, t(16;18)(p13.2;p11.2),der(18)t(16;18)(p13.2; p11.2).ish(wcp16+,18+;16-,18+)(wcp16-,18+). Now, at 36, she is again pregnant and GTG-banding and FISH-technique chromosome analysis on her amniocytes showed an apparently balanced rearrangement: 46,XX,t(16;18)(p13.2;p11.2). Couples in which both members carry similar balanced translocations are at very high risk for pregnancy loss and potentially high risk to have unbalanced offspring with malformations. There have been a few reported cases where progeny have inherited an apparently balanced translocation from a parent, yet they are mentally retarded or have malformations. The concept of imprinting has been hypothesized as a plausible explanation. For clinical purposes, it will be useful to be able to distinguish these functional differences of parental genome to avoid erroneous genetic counseling.

Program Nr: 923 from the 1999 ASHG Annual Meeting

Cytogenetic and molecular studies of an unusual presentation of Turner syndrome with 45,X/46,X,t(X;7) mosaicism. *T.W. Kurczynski[1,2], T. Durliat[2], M.A. Micale[1,2], A. Hampton[2], B. French[1], C. Gaba[1], S. Schwartz[3]*. 1) Dept of Pediatrics and; 2) Dept of Pathology, Medical College of Ohio, Toledo, OH; 3) Center for Human Genetics, Case Western Reserve University, Cleveland, OH.

A number of different karyotypes have been associated with Turner syndrome. The vast majority of these are observed in patients with a classical phenotype. In this study, we report an individual with a clinically severe presentation of Turner syndrome and an unusual karyotype. The 4lb 7oz female was the product of a 35 week gestation. At birth, a variety of dysmorphic features including downward slanting palpebral fissures, low-set ears, pectus excavatum, and bilateral simian creases were noted; however, neck webbing was not present. At age 15, this patient's medical history is remarkable for severe language delay and mental retardation, hypotonia, abnormal EEG, seizures, attention deficit disorder, thoracic scoliosis, and cerebral palsy. Initial chromosome studies in 1987 revealed a 45,X[13]/46,X,Xq+[37] karyotype. A recent peripheral blood chromosome study, including FISH analysis, more accurately defined the child's karyotype to be 45,X[7]/46,X,der(X)t(X;7)(q28;q31.1)[13]. Peripheral blood analysis of both parents revealed normal karyotypes. X inactivation studies were performed utilizing a PCR-based analysis of methylation-sensitive restriction endonuclease sites within the triplet repeat array of the androgen receptor gene. These studies revealed a skewed pattern of X-inactivation. Females with an unbalanced X autosome translocation generally demonstrate inactivation of the abnormal X, which eliminates the autosomal imbalance. If preferential inactivation of the der(X) in the 46,X,der(X) cell line occurred in this patient, one might expect that she should present a typical Turner syndrome phenotype; however, the observation of seizures, mental retardation, and other severe clinical features not typical of Turner syndrome suggests that the abnormal X may be preferentially active in at least some cells. In addition, some phenotypic features observed in this patient are consistent with partial trisomy 7 (specifically of the region 7q32®qter), but are not typical features of Turner Syndrome.

Program Nr: 924 from the 1999 ASHG Annual Meeting

Low proportion of subtelomeric rearrangements in a population of patients with mental retardation and dysmorphic features. *A.N. Lamb[1], C.H. Lytle[1], A.S. Aylsworth[2], C.M. Powell[2], K.W. Rao[2], M. Hendrickson[3], J.C. Carey[3], J.M. Opitz[3], D.H. Viskochil[3], C.O. Leonard[3], A.R. Brothman[3], M. Stephan[4], J.A. Bartley[6], M. Hackbarth[5], D. McCarthy[5], J. Proffitt[5]*. 1) Genzyme Genetics, Santa Fe, NM; 2) Univ of North Carolina Sch of Medicine, Chapel Hill, NC; 3) Univ of Utah Sch of Medicine, Salt Lake City, UT; 4) Madigan Army Med Ctr, Tacoma,WA; 5) Vysis, Inc., Downers Grove, IL; 6) Genzyme Genetics, Orange, CA.

Several recent studies have suggested that subtelomeric rearrangements in previously karyotyped individuals with mental retardation and dysmorphic features may be found in 5 - 18% of such individuals. In an attempt to verify these studies and further define the phenotypic features of the individuals that should be screened, we are undertaking a multicenter project with patients with mental retardation and dysmorphic features selected by clinical geneticists. The analysis is being performed with unique sequence subtelomeric FISH probes. All patients selected have had previous karyotype analysis, usually with high resolution banding (HRB). Currently, 43 patients have been screened by FISH and one rearrangement has been detected, for a detection rate of 2.3%. The one rearrangement is a derivative chromosome 18 that is the result of an unbalanced cryptic translocation between the distal short arm of chromosome 2 and the distal long arm of chromosome 18. Phenotyptic details and a further characterization of the size of the segments involved will be presented. A reciprocal 2;18 translocation was detected in one of the parents by FISH analysis with the relevant subtelomeric probes. The low detection rate in this study may be due to the fact that most all patients have had HRB analysis. Published studies have indicated that many of the rearrangements detected should have been detectable by HRB. Therefore, the rate of 2% in this study could reflect the rate associated with truly cryptic rearrangements and higher rates may be expected from a population of patients receiving only routine banding, in the 450 - 500 band range.

Program Nr: 925 from the 1999 ASHG Annual Meeting

Prenatal diagnosis of a familial complex chromosomal rearrangements on four chromosomes 5, 10, 16 and 18.

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We report one case of a familial complex chromosomal rearrangements (CCR) involving four different chromosomes 5, 10, 16 and 18. The CCR was detected prenatally in a fetus who was suspected of Down's syndrome by the triple test using maternal serum. Cytogenetic analysis of cultured amniotic fluid cells with GTG banding showed a 46,XX,t(5;16;10;18)(q13;q22;q11.2;q21) karyotype. Parental cytogenetic study revealed that the mother has the same CCR. RBG banding, high-resolution banding and FISH were performed to better characterize the rearranged chromosomes. No physical abnormalities were showed in the Level II ultrasonography examination at 28wks. The pregnancy is still going on.

Program Nr: 926 from the 1999 ASHG Annual Meeting

Stable dicentric duplication - deficiency chromosome 14 in a girl resulting from crossing-over within a maternal paracentric inversion. *G.F. Lefort[1], P. Blanchet[2], F. Rivier[3], A.M. Chaze[1], P. Sarda[2], J. Demaille[1, 4], F. Pellestor[4].* 1) Cytogenetics Laboratory, Hopital Arnaud de Villeneuve, Montpellier; 2) Department of Clinical Genetics, Hopital Arnaud de Villeneuve, Montpellier; 3) Department of Neuropediatrics, Hopital Arnaud de Villeneuve, Montpellier; 4) CNRS UPR 1142, Montpellier, France.

The proposita is a mentally retarded girl with dysmorphic features and multiple malformation syndrome who was presented to our Genetics Center for evaluation at the age of 32 months. She is the second child of healthy unrelated parents. Pregnancy was complicated during the second trimester by intrauterine growth retardation. No prenatal chromosome studies were done, and a 1970g baby girl (<3rd centile) was delivered at 38 weeks' gestation. On evaluation at the age of 32 months the proposita presents growth retardation, severe developmental delay, generalised hypotonia, dystonic movements, microcephaly, craniofacial dysmorphism and tapered fingers, corpus callosum hypoplasia and gastro-oesophageal reflux. The proposita's karyotype showed an elongated chromosome 14 long arm with atypical RHG-banding pattern. Subsequent studies in the parents suggested a paracentric inversion in the mother : inv(14)(q13q32.3). Characterisation of the proposita's abnormal chromosome 14 (RHG and C-banding, FISH and PRINS labeling) identified a stable dicentric duplication-deficiency chromosome 14. The occurrence of this stable dicentric chromosome 14 and the clinical duplication-deficiency features are discussed.

Program Nr: 927 from the 1999 ASHG Annual Meeting

Stable dicentric chromosomes: Four new cases and a review of the literature. *E. Lemyre[1], V.M. Der Kaloustian[2], A.M.V. Duncan[1].* 1) Dept of Pathology, Montreal Children's Hospital, McGill University, Montreal, Canada; 2) F. Clarke Fraser Clinical Genetics Unit, Montreal Children's Hospital, McGill University, Montreal, Canada.

Cytogenetically recognizable dicentric autosomes are rarely encountered as stable constitutional chromosomes in humans because of the high risk of breakage during anaphase bridge formation. However, stabilisation of the dicentric can exceptionally occur when the two centromeres are very close together (eg Robertsonian translocations) or by inactivation of one of them. We report four cases of stable dicentric chromosomes. Two of them were inherited from asymptomatic carriers: 45,XX,dic(14;18)(p11.2;p11.3) and 45,XY,dic(16;22)(p24;p11.2). The other two occured de novo in children with phenotypic manifestations of 18p deletion syndrome: 45,XY,dic(13;18)(p12;p11.2) and 45,XX,dic(13;18)(p11.2;p11.2). In our series, all cases involved an acrocentric chromosome. Only one primary constriction was seen, even though the presence of two centromeric sequences was demonstrated either by FISH studies or C-banding.This primary constriction was always on the non-acrocentric chromosome. Only 22 cytogenetically recognizable dicentrics of non homologous autosomes were reported previously. A literature review also showed a predominance of acrocentric chromosomes in stable dicentric autosomes (22/26). Only one primary constriction was found in all cells of 15/18 cases for whom this information was available. Of those, the primary constriction was the centromere of the non-acrocentric in 13/15 cases. Non-acrocentric chromosome was chromosome 18 in almost half of cases (10/22). Apart from our two patients, the dicentric was transmitted over at least two generations in only 2 families. In conclusion, our cases demonstrate further the predominance of acrocentric chromosomes in stable dicentric autosomes. Most of them will reach stability by inactivating one centromere and will be functionally monocentric. If an acrocentric is involved, its centromere is most often the inactivated one.

Program Nr: 928 from the 1999 ASHG Annual Meeting

Prenatal diagnosis of holoprosencephaly in a fetus with a recombinant 18 dup(18q)inv(18)(p11.31q11.2) mat. *N.J. Leonard[1,2], D.J. Tomkins[1,3], N. Demianczuk[4].* 1) Dept Medical Genetics, Univ Alberta; 2) Medical Genetics Clinic, UA Hosp; 3) Cytogenetics, UA Hosp; 4) Dept Obstetrics and Gynecology, Univ Alberta and Royal Alexandra Hosp, Edmonton, AB, Canada.

Holoprosencephaly was identified by ultrasonography at 18 weeks' gestation in a fetus of a G2P0A1 woman. Amniocentesis was performed and a rearrangement of chromosome 18 resembling an isochromosome for the long arm of chromosome 18 was found in all 11 colonies examined. Parental karyotypes were requested for interpretation of the rearrangement. The mother was found to have a pericentric inversion of chromosome 18 with breakpoints at p11.31 and q11.2. The karyotype of the fetus was interpreted as 46,XX, rec(18)dup(18q)inv(18) (p11.31q11.2)mat. This is the first case of a parental inversion leading to a deficiency of 18p associated with holoprosencephaly. Holoprosencephaly is a malformation complex of abnormal forebrain and midface development. It is genetically heterogeneous with associated genes localized to chromosome 21q22.3 (*HPE*1), 2p21 (*HPE*2), 7q36 (*HPE*3), 18p11.3 (*HPE*4) and 13q3.2 (*HPE*5). The critical region for holoprosencephaly on chromosome 18 has been mapped using lymphoblastoid cell lines from patients with deletions of 18p due to *de novo* anomalies or a deficiency of 18p due to segregation of a balanced translocation (Overhauer *et al*., 1995. Am. J. Hum. Genet. 57:1080). Holoprosencephaly has been described in five cases of isochromosome (18q) with additional features of the trisomy 18 phenotype (van Essen *et al*., 1993. Am. J. Med. Genet. 47:85). The presence of holoprosencephaly in these cases would be consistent with deletion of the entire 18p arm including the critical region in 18p11.3. The deficiency of the recombinant chromosome reported here represents the smallest deficiency of 18p associated with duplication of 18q. The possibility of using a FISH approach to characterize the inversion 18 is being explored.

Program Nr: 929 from the 1999 ASHG Annual Meeting

TELOMERIC ASSOCIATION IN SMOKERS EXPOSED TO LOW LEVEL OF X-RAYS. *P.E. Leone[1,2], J.C. Pérez[1], R. Burgos[1], V. Dávalos[1], M.E. Sánchez[1,2], J.C. Santos[1], C. Paz-y-Mino[1,2]*. 1) Hum Mol Genet & Cytogen Lab., Ecuadorian Catholic Univ, Quito, Ecuador; 2) Medicine Faculty, Ecuadorian Catholic University.

Deregulation of the enzyme to catalyze the telomeres replication -telomerase- could be involved in cellular immortality and oncogenesis. Alterations in telomerase activity could lead to chromosome instability, such as an increased propensity for chromosome fusions, phenomena known as telomeric association (TA). TA probably could be a chromosomal marker in populations exposed to genotoxic agents. In this study we analyzed the frequency of TA in peripheral blood of 10 smokers occupationally exposed to low levels of x-rays (1,7mSv)(SG), 10 smokers (SxRG) and 10 individuals non-smokers occupationally exposed to low levels of x-rays (1,7mSv) (xRG). These data are compared with a control group (CG). One hundred metaphases were analyzed per individual and chi-square test was used for statistical analysis. The percentage of TA in each group was 0.1% in CG, 6.0% in xRG, 9.1% in SG and 12.6% in SxRG. These results indicates that the three exposed groups show a greater percentage of TA in contrast with the CG ($p<0.001$). Also, it were observed statistical differences ($p<0.05$) to compare the three exposed groups. Because of high proportion of metaphases with TA could be considered as an indicator of malignant process, these results confirm that both x-rays and cigarette chemicals are carcinogenic agents. However, cigarette smoking might mean a greater risk for developing oncogenesis process rather than x-rays. Probably, this is a consequence of that chemical agents, such as substances present in the cigarettes smoke, are per se more closely related with malignant processes, while the action mechanism of the physic agents, such as the x-rays, not always lead to development of cancer. In addition, it suggests that there is a synergistic effect between these two agents, because of the exposed group to both agents present a greater frequency of TA. This study suggests the possibility of evaluating populations exposed to mutagens through cytogenetic analysis of peripheral blood, adding TA analysis as part of genetic instability phenomena. This work was supported by BID-111 project.

Program Nr: 930 from the 1999 ASHG Annual Meeting

Extended survey of cases with a positive diagnostic yield on second cytogenetic studies for suspected constitutional chromosome abnormalities . *K.A. Leppig[1], K.E. Opheim[1], E. Keitges[2], F. Luthardt[2], M. Lloyd[3], J. Hanna[3], S. Olson[4], E.R. Magenis[4], C.M. Disteche[5]*. 1) Children's Hosp/Reg Med Ctr, Seattle, WA; 2) Dynacare Northwest Inc., Seattle, WA; 3) Sacred Heart Medical Center, Spokane, WA; 4) Oregon Health Sciences University, Portland, OR; 5) University of Washington, Seattle, WA.

This is an extension of a preliminary study to define factors favoring a diagnostic yield for patients with a suspected constitutional chromosome abnormality (AJHG 61:A30, 1997). Over 20,000 cases from 6 cytogenetic laboratories in Washington and Oregon states completed from 1977-1998 were reviewed. Among 369 cases performed on patients with an abnormal phenotype and a normal first cytogenetic study, 49 had a "positive" diagnostic yield with an abnormal second cytogenetic study and 320 had a "negative" yield with a normal second study. Of cases with a positive diagnostic yield, 37 were non-mosaic while 12 were mosaic chromosome abnormalities. Of the non-mosaic cases, 10 were repeated within 2 years while 25 were repeated ³10 years after the initial study. All but 2 cases had structural chromosome abnormalities. The chromosome band level was ³650 in 22 cases. FISH analysis was used solely in 6 or in conjunction with G-banding in 10 cases. Seven cases had a positive family history. The repeat study was directed in 21 cases, 19 of which were correctly directed. A clinical geneticist requested the repeated study for 20 cases. Among the 12 mosaic cases, 11 were repeated within 1 year of the initial study. The mosaic abnormality was numerical in 11 cases. Most were performed on fibroblasts accounting for < 650 band level. FISH was used in conjunction with G-banding in 2 cases. Of the 7 directed cased, 6 were correctly directed. A clinical geneticist requested 5 of the repeated studies.

In this survey 49/369 (13%) cases had a positive diagnostic yield on a repeated cytogenetic study. Factors influencing the diagnostic yield included careful clinical re-evaluation, a positive family history, improved cytogenetic techniques, and evaluation of a second tissue for the detection of clinically significant mosaicism.

Program Nr: 931 from the 1999 ASHG Annual Meeting

Partial and random correction of probably familial and apparently balanced complex chromosomal rearrangements. *J.J-Y Lespinasse[1], B. Sele[2], M.R. Guichaoua[3].* 1) Laboratory of cytogenetics, General Hospital, Chambery, France; 2) Biology of reproduction laboratory, A. Bonniot Institute, Univ. of Med. La Tronche, France; 3) Biology of reproduction laboratory, Conception hospital, Marseille, France.

The authors report the case of a 38-year-old, G6, nulliparous woman. The blood karyotype showed a homogeneous complex chromosomal anomaly involving chromosomes 13, 14, 22. this anomaly corresponded to a Robertsonian translocation between one chromosome 13 and one chromosome 14. This Robertsonian translocation was in turn the subject of a reciprocal translocation with breakpoints situated between the subtelomeric extremity of 14q and the juxta centromeric part of 22q. Analysis of the siblings revealed various particularly surprising chromosomal. One of the patients sisters (G1;P0) presented a complex chromosomal anomaly perfectly identical to that presented by patient. Another sister (G4;P2) presented part of the complex chromosomal anomaly corresponding to the Robertsonian translocation between one chromosome 13 and one chromosome 14. A brother, in childless relationship, carried the other part of the complex chromosomal anomaly corresponding to a reciprocal translocation between 14q and 22q and a monosomy of the centromere of 22 and the subtelomeric extremity of 14q. This subject inherited the unbalanced form of the reciprocal translocation according to a 3:1 alternating mode. Minor psychological disorders were observed in this subject and in a paternal uncle. The family history did not reveal any other abnormalities. The mothers (G9;P6) blood karyotype was normal. The father, deceased, and two other bothers of the proband could not be studied. The chromosomes and telomeres were studied by chromosomal and molecular genetic techniques according to standard protocols. The chromosomal genetic study of this Lebanese patient and her family raises several points. Was this apparently balanced complex chromosomal anomaly inherited from the father or was there a parental germ cell mosaic? Can a spontaneous correction mechanism explain the partial and random correction of this anomaly?

Program Nr: 932 from the 1999 ASHG Annual Meeting

Detection of human sex chromosome aneuploid mosaicism by interphase FISH in noninvasively obtained and directly prepared tissues. *X. Li, J. Kobori, L. Tuttle, S. Owen, T. Tse, K. Nguyen.* Genetics Department, Kaiser Permanente Northern California, San Jose, CA.

Individuals with a mosaic sex chromosome aneuploidy may have few recognizable phenotypes and are often undetected. A cytogenetic finding of mosaic sex chromosome aneuploidy in a blood culture in those individuals poses a dilemma in genotype-phenotype correlation and for genetic counseling. Further study usually requires invasive procedures such as skin biopsy. We report here two cases who were found to have mosaic X-chromosome aneuploidies in blood lymphocytes without Turner syndrome phenotypes. Case 1 is a 35-year-old G4P1Sab2Tab1 woman who is 5'3". Case 2 is a 36-year-old G5P1Sab3 woman who is 5'7". Neither woman has a significant medical history and each has a healthy child. They were both referred for karyotyping due to a history of multiple miscarriages. Cytogenetic studies on bloods showed a 45,X[48]/47,XXX[1]/46,XX[1] karyotype in case 1 and 45,X[3]/47,XXX[1]/49,XXXXX[1]/46,XX[45] in case 2. To study the distribution of these cell lines in different tissues, cells were collected noninvasively from buccal smear and urine. Slides were directly prepared for interphase fluorescence in situ hybridization (FISH) analysis using an alpha satellite DNA probe specific for the X chromosome (DXZ1, Vysis, Inc.). Examination of 200 nuclei in total revealed different levels of X-chromosome aneuploid mosaicism in alimentary and urinary tracts in comparison with the bloods in both cases. FISH analysis in case 1 showed a much lower proportion of the X cell line but a much higher percentage of the XXX and XX cell lines in buccal smear (X=42%, XXX=47%, XX=11%) and urine (X=50%, XXX=38%, XX=12%). In case 2, FISH analysis revealed a similar proportion in buccal smear except for a higher proportion of the XXX cell line (X=6%, XXX=7%, XX=87%), but a lower percentage of the XX cell line and a higher proportion of the X and XXX cell lines in urine (X=16%, XXX=10%, XX=74%). Our findings demonstrate the direct evidence of uneven distribution of mosaic chromosome aneuploidy in vivo. Our method provides a simple, effective, and reliable tool for the study of chromosome aneuploid mosaicisms.

Program Nr: 933 from the 1999 ASHG Annual Meeting

Direct visulaization of genomic orgnization of mammalian centromeric satellite DNA families. *Y.C Li[1], C. Lee[2], S.Y. Li[1], C.C. Lin[1,3].* 1) Dept. Life Sciences, Chung Shan Medical/Dental Col., Taichung, Taiwan; 2) Dept. Obstetrics and Gynecology, Brigham and Women's Hospital, Harvard Medical School, Boston, USA; 3) Dept Laboratory Medicine and Pathology, University of Alberta, Edmonton, Canada.

The physical organization of human satellite DNAs has recently been investigated on a few centromeres at the molecular level. Multiple color FISH study with different satellite DNA probes on metaphase chromosome and extended chromatin fiber could also provide a direct visualization of genomic organization of those satellite families. Toward this approach, we have identified two mammalian centromeric satellite families (cervid satellite I and II) in caribou and Indian muntjac and performed FISH with following findings: 1. In the caribou, satellite I DNA is located on the centromeric regions of all acrocentric chromosomes. No hybridization signal was observed in a pair of submetacentric autosomes and the sex chromosomes. Satellite II DNA is located on the centromeric regions of all the chromosomes except the Y chromosome. In most acrocentric chromosomes, the satellite I signal appeared to locate more proximately to the primary constriction whereas the satellite II appeared to orient toward the short arm as two separated dots. Fiber FISH showed that satellite I and satellite II are organized in juxtaposition. The length of "beads on a string"; hybridization signals of each two satellite DNA arrays in a centromere can also be estimated. 2. In the Indian muntjac, satellite I signals were observed in the centromeric regions as well as along the chromosome arms of all chromosomes with the exception of Y chromosome. Whereas, satellite II signals were found in the centromeric region of all chromosomes. Usually, interstitial satellite I signal and satellite II signal were co-localized with some exceptions which only satellite I or satellite II signals were observed. Striking hybridization pattern was observed in the centromeric region of X+3 chromosome. A large block of green fluorescent signal of satellite II is centrally located and flanked by two blocks of red signal of satellite I DNA. This also indicates that the satellite II is ordered more toward to terminal in relation with satellite I DNA.

Program Nr: 934 from the 1999 ASHG Annual Meeting

FISH detection of low copy number HER-2/*neu* amplification in prostate cancer is more sensitive than IHC. *H.L Liu[1], R. Gandour-Edwards[3,5], R. deVere White[4,5], J.M. LaSalle[2,1]*. 1) UC Davis School of Medicine, Davis, CA; 2) Med Microbiol & Immunol Dept and Rowe Program in Hum Genetics; 3) Pathology Dept; 4) Urology Dept; 5) UC Davis Cancer Center, UCDMC, Sacramento, CA.

HER-2/*neu* oncogene amplification and overexpression occurs in 25-30% of human breast cancers and is associated with poor clinical outcome. In prostate cancers, HER-2/*neu* amplification has also been correlated with tumor progression, but detection of overexpression by immunohistochemistry (IHC) has been somewhat inconclusive in these tissues. The identification of patients with HER-2/*neu*amplification in prostate tumors is of clinical importance for identifying candidates for the drug Herceptin which blocks the HER-2/*neu* growth factor receptor. To identify HER-2/*neu*amplification in prostate cancer, paraffin-embedded archived tumor samples were tested by both IHC and fluorescence in situ hybridization (FISH). Thirty samples were selected, 15 from patients which had since shown progressed cancer and 15 from patients in stable condition. All samples were negative for HER-2/*neu* amplification by IHC performed with the DAKO kit which has been FDA approved for Herceptin® trials. In contrast, low copy number gene amplifications (3-8 signals/nucleus) were detected in 11 of 25 samples (44%) by FISH performed with Vysis HER-2/*neu* and CEP 17 probes. Cells were heterogeneous in number of FISH signals per nucleus, but most cells showing HER-2/*neu* amplification were diploid for CEP 17. In addition, a greater percentage of cells with HER-2/*neu* amplification without aneuploidy were found in progressed compared to stable patient samples, suggesting that HER-2/*neu* amplification may be an early indicator of poor prognosis in prostate cancer. Based on these results, we conclude that FISH is more sensitive than IHC for detecting low copy number amplifications of HER-2/*neu* in heterogeneous cells in prostate cancer. These results may be of significance in identifying prostate cancer patients for Herceptin® treatment in clinical trials.

Program Nr: 935 from the 1999 ASHG Annual Meeting

45,XY,der(22)t(15;22)(q13;q13.3) de novo karyotype in a child with Prader Willi syndrome. *J.C. Llerena[1,2], R.B. da Silva[3], H. Ramos[1], E. Bastos[2], V.E. Ferraz[4], J.M. Pina Neto[4], J.C. Cabral de Almeida[1,2].* 1) Dept de Gen(é)tica M(é)dica, IFF/FIOCRUZ, Rio de Janeiro, Rio de Janeiro, Brazil; 2) Unidade de Citogen(é)tica Humana/IBCCF/UFRJ; 3) Servi(ç)o de Gen(é)tica/HUPE; 4) Dept. Gen(é)tica/Faculdade de Medicina Ribeir(ã)o Preto/USP.

Rare chromosomal rearrangements associated to Prader Willi syndrome (PWS) have been reported in 5% of patients frequently associated to unbalanced translocations involving the critical 15q11-q13 PWS/AS region. As a result, loss of non-imprinted paternal genes has been identified through SNRPN molecular studies. We report the case of a 2 years old infant with severe hypotonia, poor sucking reflexes at birth, and a G- band karyotype 45,XY, der (22)t(15;22) (q12~13;q13.3),-15 de novo. FISH investigation using probes (ONCOR) SNRPN, GABRB3, 22q11.2 (D22S75) and 22q13.3 (D22S39) showed that the derivative chromosome was composed of chromosome 22 (two copies of 22q11.2 and 22q13.3 probes) and no hybridization signal was observed with both SNRPN and GABRB3, however chromosome 15 distal control probes were detected. The investigation for intercalary telomeric sequences on the der22 by FISH did not yield reliable results. Thus, the patients karyotype by FISH was defined as 45,XY.ish der(22)t(15;22)(q13;q13.3) de novo.(SNPRN-, GABRB3-)(D22S75x2, D22S39x2). Clinical examination of the propositus at 2 years showed heigth and weigth below 25th centile, normal cephalometric measures, triangularly shaped face, supracilliary hemangiomas, almond shaped eyes, convergent strabism, high and narrow palate, normal male external genitalia, acromicria and motor and mental retardation. To our knowledge 28 cases of PWS and unbalanced translocation have been reported and in 25 of them the abnormality was de novo and paternal in origin, as also demonstrated in our case by molecular studies (D15S113,D15S11,GABRB3), as expected.

Program Nr: 936 from the 1999 ASHG Annual Meeting

De novo bisatellited chromosome 22 in a patient with mild mental retardation. *L.A. Lopez Miranda, P. Barbero, M. Mollica, A. Rebechi, I. Aranda, L. Alba.* Centro Nacional de Genetica Medica, Buenos Aires, Argentina.

We described a 7 year old girl with a karyotype 46,XX,22qs who was referred to our center because of mild mental retardation. The proband is the fourth child of healthy, non-consanguineous parents. She was delivered at 40 weeks of gestation from a 36-year-old mother and a 44-year-old father. There is no relevant genealogical background. Weight and height were at the 75th centile, and head circumference at the +1SD for her age. She showed downslated palpebral fisures, hypermetrophy, cup shaped and prominenet ears. Echocardiography and brain CT scan were normal. Cytogenetic studies using G; C and NOR banding techniques were performed. An abnormal bisatellited chromosome 22 was detected. DA/DAPI did not showed bright fluorescence on the long arm of this derivative chromosome. FISH using chromosome 22 painting probe(WCP22) revelead that the euchromatic material was 22 on the abnormal chromosome and with a centromeric 14/22 probe (D14Z1/D22Z1) showed only one 14/22 centromeric region on this chromosome. FISH analysis using centromeric 13/21 probe (D13Z1/D21Z1) are in progress. Parental karyotypes were normal. The abnormal bisatellited chromosome must have been originated by a reciprocal translocation between the long arms of a chromosom 22 and the short arms of an acrocentric chromosome leading to a terminal deletion 22q. The clinical findings in our patient are similar to those previously described for 22q deletions.

Program Nr: 937 from the 1999 ASHG Annual Meeting

Multiple second trimester maternal serum markers as predictors of preeclamptic pregnancies. *D.A. Aitken[1], S. Bergh[2], J.A. Crossley[1], S. Lahiff[1], R. Arngrimsson[2], J.M. Connor[1].* 1) Biochemical Genetics Div, Inst Medical Genetics, Glasgow, Scotland; 2) University and National Hospital, 101 Reykjavik, Iceland.

Preeclampsia is a condition associated with the third trimester of pregnancy when it may seriously threaten the well-being of mother and fetus. Recognition of preeclampsia is usually delayed until onset of the symptoms and a predictive test in early pregnancy would be valuable. We have investigated the levels of seven feto-placental markers in second trimester maternal serum from 75 women who subsequently developed preeclampsia at later gestations. All had screening for neural tube defects and Downs syndrome between 15 and 20 weeks gestation when alphafetoprotein (AFP) and human chorionic gonadotrophin (ihCG) were analysed. Serum samples from cases and controls (n = 250) were recovered from frozen storage and assayed retrospectively for dimeric inhibin A (InhA), free beta hCG (FbhCG), pregnancy specific 1 glycoprotein (SP-1), pregnancy associated plasma protein A (PAPP-A) and cancer antigen 125 (CA125) using specific immunoassays. Analyte levels for each marker were converted to multiples of the appropriate gestational median (MOM) derived from the control series. Significantly elevated median MOM levels (Mann whitney $p<0.01$) were found for AFP (1.19 MOM), ihCG (1.31 MOM), and InhA (1.36 MOM) in the preeclamptic pregnancies. PAPP-A levels were reduced (0.76 MOM) but this did not reach statistical significance ($p = 0.56$) and there was no significant difference in levels of SP-1 (1.06 MOM) or FbhCG (1.09 MOM). In this study the increase in InhA level in preeclampsia was less marked than that reported in two previous studies (Muttukrishna et al, Lancet 1997, 349:1285; Cuckle et al, BJOG 1998, 105:1101). However, identification of women at risk may be improved by the use of additional markers (eg. AFP and ihCG) allowing clinical monitoring to be started early which, in turn, may lead to improved management of pregnancies and the development of effective treatment.

Program Nr: 938 from the 1999 ASHG Annual Meeting

A unique mosaic marker chromosome resulting from a familial Robertsonian translocation (21;22). *S. Arab[1], D. Chitayat[2,5], A. Gardner[3], E. Winsor[4,5].* 1) pathology and laboratory Medic, The Hospital for Sick Children, Toronto, Ontario, Canada; 2) Division of clinical Genetics, Hospital for sick children; 3) Genetic services, Likeridge Health Oshawa, Ontario; 4) Toronto General Hospital, Depat. Laboratory Medicine and pathobiology; 5) The prenatal diagnosis program University of Toronto, Canada.

A mosaic marker chromosome found in amniotic fluid was shown to be the result of deletion of a segment of a maternally inherited Robertsonian 21;22 translocation. Using a DiGeorge/velocardiofacial FISH probe (Vysis), it was demonstrated that in metaphases with the marker present there was a probe signal on the marker, but no signal on the translocation chromosome. However, in the absence of the marker there was a probe signal on the translocation chromosome. The karyotype was 45,XY,der(21;22)(q10;q10)/46,XY,der(21)(pter->21q10::22q11.2->qter),der(22)(22q10->22q11.2). Centromeric fission of the dicentric translocation chromosome resulted in two separate functional centromeres. Although the location of the second breakpoint for this marker is not known precisely, we speculate that the break may have occurred in the same "hot spot" which results in deletion of the DiGeorge/velocardiofacial region. Since no marker was found in maternal cells, it is suggested that the deletion and marker formation probably occurred post zygotically in the fetus. Fetal ultrasound at 19.5 weeks revealed a single fetus with measurements consisted with gestational age. Autopsy revealed a male fetus with no detectable abnormalities except for a small thymus. This case illustrates the difficulty in estimating risk of fetal abnormalities associated with de novo marker chromosomes. Marker chromosomes are usually assumed to contain "extra" material and the risk of associated fetal abnormalities is usually related to the amount of euchromatin present in the marker. This unique example illustrates that a marker chromosome which was initially assumed to be extra may be part of a balanced karyotype.

Program Nr: 939 from the 1999 ASHG Annual Meeting

Sex chromosome complement of placental trophoblast in X chromosome aneuploid pregnancies. *I.J. Barrett[1], W.P. Robinson[2], D.K. Kalousek[1]*. 1) Dept of Pathology,; 2) Dept of Medical Genetics, Univ of BC, Vancouver, Canada.

Monosomy X is seen in approximately 11% of spontaneous abortions. The fetal phenotype of 45,X consists of cystic hygroma, hydrops, pulmonary hypoplasia, horseshoe kidney, congenital heart defects. Less than 1% of 45,X conceptuses survive intrauterine development. It has been shown that approximately 5% of trisomy 13 and 18 conceptuses survive to third trimester and in these a significant level of disomy 13 or 18 has been shown to occur specifically in the trophoblast of the placenta. The role of chromosomal diploidy in placental trophoblast in gestations with 45,X fetuses has not been studied. The present study determines the distribution of disomy X in trophoblast and various tissues of conceptuses with prenatally diagnosed mosaicism for X chromosome aneuploidy or non-mosaic 45,X pregnancies. Eighteen pregnancies with X chromosome aneuploidy were analysed for presence of disomy X in the trophoblast. The gestational ages ranged from 14 to 40 weeks. Prenatal diagnosis using traditional cytogenetic methods had been performed in 15 of them; 5 involved X chromosome mosaicism and 10 non-mosaic 45,X. In addition 3 were terminated following identification of cystic hygroma with post-termination cytogenetic analysis demonstrating non-mosaic fetal aneuploidy 45,X. Cytogenetic analysis (direct, FISH or culture) was performed on trophoblast, chorionic stroma and fetal tissues if available. In all 5 cases with fetal mosaicism for X chromosome the trophoblast showed high levels of disomy X by FISH with varying levels in other tissues studied. Disomy X was observed in the trophoblast in only 3 out of 13 pregnancies with non-mosaic 45,X fetuses. The presence of high levels of disomy X in trophoblast in cases with fetal mosaicism suggests that these pregnancies originated as a diploid conceptus and the loss of an X chromosome occurred after trophoblast differentiation. Intrauterine survival of non-mosaic 45,X fetuses does not appear to be dependent upon the presence of disomy X in the trophoblast. DNA analysis of the parental origin of disomy X in the trophoblast of mosaic and non-mosaic 45,X pregnancies is in progress. ¥.

Program Nr: 940 from the 1999 ASHG Annual Meeting

A novel *SOX* gene expressed in the developing dermomyotome and neural crest. *K.M. Bell, A.H. Sinclair.* Department of Paediatrics, University of Melbourne, Centre for Hormone Research, Royal Children's Hospital Melbourne, Victoria, Australia.

SOX genes are members of a large and highly conserved family encoding putative transcription factors related by a common DNA binding domain, the HMG box. Twenty seven mammalian *SOX* genes have been identified to date. *SOX* genes have a restricted spatial and temporal expression profile in the developing embryo. Three members of the *SOX* gene family have been implicated in human diseases. Mutations in *SRY, SOX9* and *SOX10* cause XY sex reversal, Campomelic Dysplasia and Hirschsprung-Waardenburg syndrome respectively. Further study of *SOX* genes will elucidate their role in normal development and human disease.

In order to isolate novel *SOX* genes we screened a day 5 and 6 chick urogenital embryonic cDNA library with known *SOX* genes. We identified a novel *SOX* gene with highest sequence homology to the *SOX* subgroup which includes *SOX9* and *SOX10*. Using whole mount *in situ* hybridisation we first detected expression in early day two embryos. The temporal and spatial expression profile suggests a role in the development of the dermomyotome and neural crest cells, as well as some of the derivatives of these tissues. Expression is also seen in discrete bands in the ventricular zone of the neural tube.

Further studies mapping the human orthologue will help to identify possible congenital defects involving this gene. It might be expected that mutations in this gene will disrupt normal muscle and neural crest development.

Program Nr: 941 from the 1999 ASHG Annual Meeting

Abnormal maternal serum inhibin-A levels in trisomy 16 mosaic pregnancies. *P.A. Benn, R. Collins.* Div Genetics, Dept Pediatrics, Univ Connecticut Hlth Ctr, Farmington, CT.

Prenatal diagnosis of trisomy 16 mosaicism is associated with fetal death, IUGR, preterm delivery, and diverse congenital anomalies. Second trimester maternal serum alpha-fetoprotein (MS-AFP) levels are often high and, in addition, human chorionic gonadotropin (hCG) concentrations (and therefore Down syndrome (DS) risk) can be substantially elevated (Benn, AJMG,79,121;1998).

We analyzed Inhibin-A (Inh-A) levels in thawed maternal serum specimens from three previously reported cases with confirmed trisomy 16 mosaicism. Each pregnancy was associated with IUGR, preterm delivery and/or fetal anomalies. Case 1 (with triple test results MS-AFP= 2.80 MoM, hCG= 12.02 MoM, uE3= 0.81 M0M, and DS risk of 1:55) had Inh-A= 11.40 MoM and a DS risk based on all four analytes of 1:15. Case 2 (MS-AFP= 1.64 MoM, hCG= 21.41 MoM, uE3=0.59 MoM, and triple test DS risk 1:24) had Inh-A =13.32 MoM with a quad DS risk of 1:5. Case 3 (MS-AFP= 4.98 MoM, hCG= 8.62 MoM, uE3= 0.75 MoM, triple test DS risk of 1:106) had Inh-A= 9.90 MoM and a quad DS risk of 1:34. Inh-A was also measured in a fourth specimen from a pregnancy with IUGR, preterm delivery and fetal anomalies consistent with trisomy 16 mosaicism but normal amniotic fluid cytogenetic analysis (occult mosaicism). This case (with triple test results MS-AFP= 2.13 MoM, hCG= 13.32 MoM, uE3= 0.66 MoM, and DS risk 1:16) had Inh-A= 8.48 MoM and a quad DS risk of 1:2.

This preliminary data therefore indicates that very high Inh-A levels are often present. Based on simulation using published means, standard deviations and correlation coefficients, less than 1 in 100,000 normal and less than 0.14% of DS pregnancies will have MS-AFP>1.00 MoM, hCG>5.00 MoM and Inh-A>5.00 MoM. This pattern would therefore appear to be useful in identifying trisomy 16 mosaicism (and possibly other disorders with similar placental disturbance). We hypothesize that the degree of elevation of the analytes will reflect the relative proportion of abnormal cells present in the placenta and that the most extreme values will be associated with the greatest risk for poor pregnancy outcome.

Program Nr: 942 from the 1999 ASHG Annual Meeting

Declining termination rates for chromosome abnormalities identified prenatally by amniocentesis in southeast United States. *R.G. Best[1], A.M. Sanders[1], K.C. Phelan[2], V.A. Vincent[1]*. 1) Dept Obstetrics/Gynecology, Univ South Carolina Sch Med, Columbia, SC; 2) Greenwood Genetic Center, Greenwood, SC.

We report the study of pregnancy termination decisions following the prenatal diagnosis of cytogenetic abnormalities by amniocentesis among women tested in the southeast United States from 1987 through 1998. A total of 679 chromosome abnormalities were identified from 28,690 pregnancies (2.4%) at six centers. This includes 269 cases of trisomy 21, 126 sex chromosome abnormalities, 113 cases of trisomy 18, 38 cases of trisomy 13, 35 balanced de novo rearrangements, 24 unbalanced structural rearrangements, and 74 others. Outcome data were obtained from 419 cases excluding spontaneous losses and cases identified after the limits for termination. The overall termination rate was 57% with higher rates of termination associated with greater phenotypic severity. Termination rates were highest for trisomy 13 (86%), followed by trisomy 18 (74%), trisomy 21 (68%), unbalanced rearrangements (65%), sex chromosome abnormalities (25%), and balanced de novo rearrangements (8%). Termination rates also correlated with race (p=.0353). A trend was apparent between termination rates and maternal age (p=0.26) with rates of 44%, 54%, and 62% in women aged <25, 25-34, and >35 respectively. The most common indications for testing among all abnormalities identified include advanced maternal age (32%), abnormalities on ultrasound (27%), positive serum screening (22%), and combined AMA/ positive screen test (10%).Our findings represent a higher frequency of chromosome abnormalities, and an overall lower termination rate compared with an earlier study of termination rates among prenatally diagnosed cytogenetic abnormalities in the southeast US (Vincent et. al, 1991). The previous study revealed 416 cytogenetic abnormalities among 26,950 pregnancies (1.5%) studied during the period from 1969-1986. The overall termination rate of 73% included 95% of trisomy 13 and 18 pregnancies, 92% of trisomy 21, and 42% of sex chromosome abnormalities. The distribution of indications revealed 75% referred for AMA, 7% with abnormal ultrasound, 4% for abnormal serum screening in that study.

Program Nr: 943 from the 1999 ASHG Annual Meeting

Isolating fetal cells from maternal blood: Strategies to increase sensitivity for fetal aneuploidy detection. *F.Z. Bischoff[1], D. Lewis[2], J.L. Simpson[1,3], D.D. Nguyen[1], D. Marquez-Do[1], A. Bryson[2], J. Scott[2], K. Leonard[1], S. Elias[4]*. 1) Depts OB/GYN; 2) Immunology/Microbiology; 3) and Molecular/Human Genetics, Baylor College of Medicine, Houston, TX; 4) Dept OB/GYN, Univ of Illinois, Chicago.

Increasing numbers of investigators are pursuing strategies for isolation and analysis of fetal cells in maternal blood as a method of non-invasive prenatal diagnosis. We have previously described methods for enrichment (Cytometry 23:218 1996) and aneuploid detection (Am J Obstet Gynecol 179:203 1998). Our efforts have focused on obtaining consistency in flow-sorting methodologies and optimizing FISH analysis for aneuploidy detection. POPULATION-Blood samples were obtained from women seen for prenatal genetic diagnosis at Baylor (n=389) and Wayne State (n=42) (14 wks mean gestational age). Of 431 samples, 354 were enrolled in a multicenter NICHD study; 77 non-enrolled cases were analyzed similarly. METHODS-A variety of enrichment strategies have been employed, namely positive selection for glycophorin A (gly A), CD71 (transferrin receptor) or gamma globin (HbF) and negative selection for CD45. Following enrichment, antibody-stained cells are fixed in formaldehyde and permeabilized with 0.1% Triton-X prior to sorting. RESULTS-Each of five gly A antibodies produced agglutination, resulting in our abandoning gly A for positive selection. Positive selection by flow-sorting for either $CD71^+$ or gamma-$globin^+$ cells seems equally successful. Specific modification of cellular permeabilization strategies have now maximized accessibility of DNA to probes; cellular fixation after enrichment and storage conditions also affect cellular integrity for subsequent FISH. ANEUPLOID DETECTION (1999)-Overall, we detect 42% of fetal aneuploid cases with a very low false-positive rate - 0.5% for trisomies 13, 18 and 21, respectively. CONCLUSION-Our goal of greater aneuploid detection, at least that of maternal serum screening and nuchal translucency, is not yet achieved. Alternative methods of enrichment (i.e. MACS or density gradient separation) are being investigated to improve efficiency in fetal cell recovery. NICHD N01HD43203.

Program Nr: 944 from the 1999 ASHG Annual Meeting

Isolation of fetal nucleated red blood cells (FNRBCs) from maternal blood cultures: Optimization of culture conditions for flow sorting based on correlated cellular HbF and HbA contents. *R.M. Bohmer, D.K. Zhen, D.W. Bianchi.* Division of Genetics, Dept Pediatrics, Tufts University School of Medicine, Boston, MA.

Background: Using flow cytometric measurement of correlated cellular HbF and HbA contents, we have shown previously that FNRBCs in culture develop a hemoglobin profile that makes them distinguishable from most adult erythroid cells, so that FNRBCs can be detected and highly enriched from maternal blood cultures (Br J Haematol 1198;103:351). However, some adult cells make HbF (F+ cells) like fetal cells, which limits the purity of fetal cell isolation by flow cytometry.

Methods: In peripheral blood cultures from pregnant women, we tested a variety of drugs and culture conditions for their ability to selectively suppress the growth of adult F+ cells. Analysis was performed using two-color flow cytometry, FACS and FISH.

Results: The following cytokines or drugs had no effect on the proportions of adult F+ cells: TNFalpha, Mip1alpha, Activin A, SDF-1alpha, PGE2, dexamethasone, IFNgamma, IGFI and IGFII. The proportions of adult F+ cells were strongly increased by TGFbeta. Two modifications of standard culture conditions were found to suppress the growth of adult F+ cells dramatically while having little effect on fetal F+ cells: the use of charcoal-treated human umbilical cord serum at low concentrations, and the omission of IL-3 from the standard cytokine cocktail. Under these conditions, the enrichment of FNRBCs in co-cultures of fetal and adult blood cells was estimated to approach 1000 fold.

Conclusions: The biology of fetal and adult erythroid cells in culture is complex. However, culture conditions can be designed to suppress the growth of adult F+ cells selectively, permitting a dramatic enrichment of fetal nucleated red cells in erythroid cultures from maternal blood. We will test this procedure on blood samples obtained from pregnant women carrying normal and abnormal fetuses.

Program Nr: 945 from the 1999 ASHG Annual Meeting

Normal twin pregnancy following ICSI of ejaculate spermatozoa from a patient with Klinefelter's syndrome. *N. Bolduc, M.M. Bielanska, S.L. Tan, A. Ao, V. Desilets.* McGill University Health Centre, Royal Victoria Hospital, Montreal, Quebec, Canada.

Klinefelter's syndrome is usually associated with azoospermia or severe oligospermia. Recent evidence suggests that 47,XXY germ cells are able to complete meiosis and produce sperm nuclei. In recent years, pregnancies and births have been reported following intracytoplasmic sperm injection (ICSI) using spermatozoa collected through testicular biopsy.

We report a twin pregnancy in a couple in which the man has Klinefelter's syndrome. Motile spermatozoa were present occasionally in the ejaculate of this man. His blood karyotype, done twice in different laboratories, was 46,XY(1)/47,XXY(59) and 47,XXY(50). FISH analysis of his sperm revealed a normal number of sex chromosomes in 94.4% of the cells. They underwent ICSI without testicular sperm extraction. Due to technical difficulties, preimplantation genetic diagnosis failed. Three embryos were transferred resulting in a dichorionic, diamniotic twin pregnancy. At 12 weeks, normal nuchal translucency measurements were obtained in both twins. At 16 weeks, amniocentesis showed a 46,XX karyotype in each twin. No fetal anomalies were seen at the 18 week ultrasound.

To our knowledge, this is the second report of a successful pregnancy using spontaneous spermatozoa from ejaculate of a man with Klinefelter's syndrome. Patients with Klinefelter's may achieve pregnancy through reproductive technology without having testicular biopsy. Preimplantation diagnosis and/or prenatal diagnosis should be considered. It is likely that this patient has 46,XY/47,XXY gonadal mosaicism. We are in the process of completing further cytogenetic analysis in him.

Program Nr: 946 from the 1999 ASHG Annual Meeting

Mouse Sry requires a CAG repeat domain for male sex determination. *J. Bowles, L. Cooper, J. Berkman, P. Koopman.* CMCB, University of Queensland, Brisbane, Queensland, Country.

SRY, the mammalian Y-chromosomal sex-determining gene, encodes a protein characterized by a DNA-binding and bending domain referred to as the HMG box. Despite the pivotal role of this gene, only the HMG box region has been conserved through evolution, suggesting that function depends solely on the HMG box and therefore that SRY acts as an architectural transcription factor. In mice (genus Mus), Sry also includes a large CAG trinucleotide repeat region encoding a C-terminal glutamine-rich domain that can act as a transcriptional trans-activator in vitro. However the absence of this or any other potential trans-activating domain in other mammals has raised doubts as to its biological relevance. To test directly whether the glutamine-rich region is required for SRY function in vivo, we created truncation mutations of the Mus musculus musculus Sry gene and tested their ability to induce testis formation in XX embryos using a transgenic mouse assay. Sry constructs that encode proteins lacking the glutamine-rich region were unable to effect male sex determination, in contrast to wild-type counterparts. We conclude that the glutamine-rich repeat domain of the mouse SRY protein plays an essential role in sex determination in vivo, and that SRY may act via a fundamentally different biochemical mechanism in mice compared to other mammals.

Program Nr: 947 from the 1999 ASHG Annual Meeting

Tissue specific involvement in fetal trisomy 16. *H. Bruyere[1,2], I.J. Barrett[2], D.K. Kalousek[2], W.P. Robinson[1]*. 1) Dept. of Medical Genetics; 2) Dept. of Pathology, University of British Columbia, Vancouver, Canada.

Placental mosaicism is found in 1-2% of viable pregnancies diagnosed by chorionic villous sampling and is typically confined to the placenta. However, in about 10% of these cases, follow-up amniocentesis reveals fetal involvement. The extent and tissue specific distribution of the trisomy in the fetus is expected to depend on the timing of the responsible event, the chromosome involved, the role of selection against the trisomy in different tissues or stochastic factors. To understand how mosaicism may be distributed in fetal tissues, we have performed fluorescence in situ hybridization and/or DNA marker analysis in fetal/neonatal tissues in 7 cases of mosaic trisomy 16. Four cases were terminated after a positive AF (13 to 50% trisomy). Case 1 was trisomy positive in skin but negative in brain, spleen, kidney. Case 2 was trisomy positive in lung but negative in skin, kidney, brain, spleen, liver, adrenal, thymus, gonad and amnion. Case 3 was trisomy positive in lung but negative in kidney. Case 4 was negative for trisomy in thymus, blood and amnion. Trisomy 16 was diagnosed in case 5 on CVS. Despite a normal AF result, in utero death occurred at 24 weeks and trisomy was confirmed in brain but not in lung. Case 6 (previously published) was terminated after a positive CVS result. Oocytes and amnion were positive for the trisomy but skin, lung, kidney, brain and blood were negative. In case 7, trisomy 16 mosaicism was identified in the placenta and in spleen after neonatal death of a phenotypically abnormal baby. Amnion and blood were diploid. These results suggest that 1) trisomy 16 detected by AF is frequently confined to a single or a few fetal tissues. The affected tissues can differ from case to case, possibly explaining the large phenotypic variability in liveborn infants with mosaic trisomy 16. 2) AF results do not reflect the level of trisomy in affected fetal organs. 3) AF may reflect the presence of trisomy in skin, lung, kidney or amnion, tissues which contribute to AF. However, involvement of other tissues cannot be predicted by AF analysis and "occult" fetal mosaicism may occur in some cases diagnosed as "confined placental mosaicism".

Program Nr: 948 from the 1999 ASHG Annual Meeting

Prenatal detection and follow-up of Renal Cystic Disease. *L. Carrillo[1], R. Sanchez[1], C. Llanusa[1], J. Oliva[1], L. Rodriguez[1], L. Heredero[2].* 1) Genetics, Gonzalez Coro Hospital, Havana City, Havana City, Cuba; 2) National Center of Genetics.

The prevalence at birth of urinary tract anomalies is 3 per 1000 liveborns. Since 1990 the genetics unit at the Gonzalez Coro hospital in Havana City conducts prenatal ultrasound diagnosis of renal anomalies with the goals of improving their prenatal detection, delineating their natural history and developing guidelines for their management after birth. Between 1990 and 1997, 34 cases of unilateral or bilateral renal cystic lesions were detected in the second trimester of gestation. In 9 of these, the parents decided the termination of pregnancy: these cases tended to be bilateral, progressive, and/or associated with oligohydramnios or additional malformations. In all cases the pathologic examination after termination confirmed the prenatal ultrasound diagnosis: single renal cyst (1), infantile polycystic kidney (4), multicystic kidneys (3) and obstructive cystic disease (1). Of the 25 cases born at term, postnatal ultrasound confirmed the same lesion detected prenatally: unilateral multicystic kidney (15), bilateral polycystic kidney (1) and single renal cyst (1). In 6 cases, the postnatal tests determined a renal lesion different from the one predicted prenatally and in 2 cases the postnatal studies failed to show any renal anomaly. Of the 23 patients born at term with a renal anomaly, only 2 required unilateral nephrectomy and 3 required occasional antibiotic treatment and the remaining did not require any treatment. In 13 of the 15 patients born with unilateral multicystic kidney, the affected kidney eventually atrophied. Overall, of the 34 cases detected prenatally, the diagnosis after termination or birth coincided exactly in 26 cases (76.5%), and partially (renal anomaly different from the one diagnosed prenatally) in 6 cases (17.6%). Only 2 cases representred true false positives.

Program Nr: 949 from the 1999 ASHG Annual Meeting

Cytogenetic and FISH analysis in two prenatal cases with half cryptic chromosome rearrangements involving a terminal 2q37 deletion. *S.W. Cheung[1], C.M. Lese[2], J.L. Smith[1], D.A. Kass[3], C.G. Hatjis[3], J.C. Sawyer[1], D.H. Ledbetter[2]*. 1) Laboratories Genetic Services, Houston, TX; 2) Dept.of Human Genetics, Univ.of Chicago, Chicago, Ill; 3) Perinatal Services, St. Agnes Healthcare, Baltimore, MD.

Terminal 2q37 deletion is a recognizable syndrome with growth retardation, developmental delay, microcephaly, dysmorphic features, cardiac defects, genital anomalies, and syndactyly/clinodactyly. We report two novel cases with subtle terminal deletions of 2q37 resulting from paternal chromosome rearrangements which became apparent with the aid of telomeric probes. Amniocentesis was performed in case 1 due to an abnormal trisomy screen (risk for Down syndrome 1 in 37). Chromosome analysis revealed 46,XX,add(2)(q37). The paternal karyotype was 46,XY,inv(2)(p25.1q37.2). FISH studies using both centromeric and 2p telomeric probes (ONCOR) were performed on fetal and paternal cells. The result confirmed the inversion in the father and showed that the fetus carried a rec(2) with a partial duplication of 2p at band 25.1 and monosomy for 2q at band q37. Pregnancy termination was elected and abnormalities were noted in the abortus. Amniocentesis was performed in case 2 for advanced maternal age and abnormal trisomy screen (risk for Down syndrome 1 in 213). Initial results were 46,XY,add(2)(q27), which was also found in the father. FISH analysis carried out with a probe specific for the 2q telomere (BAC 172113) revealed monosomy 2q in the amniotic fluid sample. FISH analysis using the same probe on the father identified a balanced translocation between 2q and 11p, a translocation which was only detectable by cytogenetics after the FISH result was available. These results verify paternal transmission of the unbalanced translocation in the amniotic fluid sample. Pregnancy termination was elected; no follow up studies are available. Detailed cytogenetic studies for chromosome rearrangements, FISH analysis and follow up studies will be presented.

Program Nr: 950 from the 1999 ASHG Annual Meeting

Prenatal diagnosis of glutaric aciduria type II associated with cystic renal changes and elevated maternal serum alpha-fetoprotein. *C.A. Chisholm[1], F. Vavelidis[1], M.A. Lovell[1], D.S. Roe[2], C.R. Roe[2], L. Sweetman[2], W.G. Wilson[1]*. 1) Depts. of Obstetrics and Gynecology, Pathology, and Pediatrics, Univ of Virginia Health System, Charlottesville, VA; 2) The Institute of Metabolic Disease, Baylor Univ Medical Center, Dallas, TX.

We report the occurrence of glutaric aciduria type II (GA II) in two consecutive pregnancies in a young Caucasian non-consanguineous couple. In the first pregnancy, the maternal serum alpha-fetoprotein (MSAFP) value was elevated (2.62 MOM). Ultrasound showed a fetus two weeks smaller than menstrual dates with enlarged, echogenic, microcystic kidneys and oligohydramnios. The couple elected to terminate the pregnancy because of concerns about severe cystic renal disease. Post-mortem examination of the fetus showed glomerulocystic renal changes and hepatic steatosis. Fibroblast analysis confirmed electron transfer flavoprotein deficiency (GA II). In the second pregnancy, ultrasound at 15 weeks showed an early growth lag and kidneys that were normal in size and appearance. Results of testing included an elevated MSAFP (3.4 MOM), elevated amniotic fluid AFP (2.51 MOM), normal female karyotype, negative acetylcholinesterase, elevated amniotic fluid C4, C5, and C5-diCOOH acylcarnitines, and increased C5/C3 and C5-diCOOH/C3 acylcarnitine ratios by tandem mass spectroscopic analysis. Subsequent in vitro studies of fat oxidation in cultured amniocytes were consistent with GA II. Follow-up ultrasound showed enlarged, echogenic, microcystic kidneys. The couple elected to terminate the pregnancy, and the findings were confirmed on post-mortem examination. These cases provide additional information regarding the evolution of renal changes in fetuses affected with GA II, as well as showing a relationship with elevations in AFP, which may be useful in counseling the couple at risk. GA II should be considered in the differential diagnosis of elevated AFP and cystic renal disease in the fetus. Early fetal growth delay may be an additional finding.

Program Nr: 951 from the 1999 ASHG Annual Meeting

Short Tandem Repeats polymorphism Is Useful in Diagnosis of Trisomy 21. *D.C. Chu[1,3], L.R. Boots[2], C.L. Wu[3], K.C. Tsao[3], D.T.Y. Chiu[1], C.F. Sun[3].* 1) School of Medical Technology, Chang Gung University, Tao Yuan, Taiwan; 2) Department of OB/GYN, University of Alabama at Birmingham, USA; 3) Department of Clinical Pathology, Chang Gung Medical Center, Lin-Kou, Taiwan.

Human chromosome 21, the smallest human chromosome, constitues approximately 1.2% of the human genome. A large number of sequence-tagged sites (STS)and polymorphic DNA markers have been identified on the human chromosome 21 recently. Since quite a number of trisomy 21 cases result from nondisjunction during meiosis I, it was hypothesized that the 3 chromosome 21s of different parental origins could be differentiated by their DNA polymorphism.

Forty-eight amniotic fluid samples from pregnancies complicated with fetal Down syndrome were analyzed for D21S11 and interferon-a receptor (IFNAR)- intervening sequences. These loci contain tetranucleotide repeat sequences. The heterogeneity rates of these 2 markers were reported to be 0.90 and 0.83, respectively. Polymerase chain reaction (PCR) was performed to amplify the sequences and the band patterns were resolved by polyacrylamide electrophoresis with ABI Prism 377.

The preliminary data revealed that 35 of 40 (87.5%) fetal Down syndrome cases analyzed for IFNAR showed 3 distinctive bands, each band represents one individual chromosome 21, while 24 of 30 (80%) fetal Down syndrome cases analyzed for D21S11 showed 3 distinctive bands. Altogether 39 of 40 (97.5%) of the fetal Down syndrome cases studied showed 3 distinctive bands.

In conclusion, polymorphic DNA markers are useful in determining the number of chromosome 21 present in the fetal cells. The high sensitivity of the preliminary data suggested possible clinical application of this technique for prenatal diagnosis of chromosome aneuploidy.

Program Nr: 952 from the 1999 ASHG Annual Meeting

Carnitine Acylcarnitine Translocase Deficiency : First prenatal genetic analysis on crude chorionic villus biopsy.

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Carnitine acyl carnitine translocase (CACT) deficiency is a severe disorder of long-chain fatty acid oxidation with a bad prognosis. For the last five years, we performed 8 prenatal diagnosis in 6 families by biochemical investigations on cultured trophoblasts or amniotic cells (fatty acid oxidation assays and CACT activity measurements). These methods are accurate but the diagnosis was only obtained in 30 to 40 days. Cloning and sequencing of human CACT cDNA have allowed the beginning of mutation recognition in patients. The recent determination of the exon-intron structure of the human CACT gene has opened the way to direct analysis of fetal DNA for a faster prenatal diagnosis of CACT deficiency. We have identified CACT mutations in 9 unrelated families by directly sequencing the entire cDNA and confirming the mutational status on genomic DNA. In one of these families, two different mutations, a C-G transversion in exon 7 leading to a P751R missense mutation inherited from the father, and a G-A transition in the splice acceptor site in intron 6 inherited from the mother were found, before initiation of a second pregnancy by the mother. Fetal genomic DNA was extracted from a chorionic villus biopsy sampled at the 12th week of gestation. Paternal and maternal contribution were checked with highly polymorphic microsatellite markers. Intronic oligonucleotides primers were used to amplify both the exon 7 and the associated splice donor and acceptor sites. Fetal DNA sequence analysis indicated that the fetus had received both normal CACT alleles. With this approach, the diagnosis was obtained within 2 days. Long chain fatty acid oxidation assays and CACT activity measurements, performed on cultured trophoblasts and amniocytes confirmed, one month later, that the fetus was not affected. Supported by Assistance Publique de Paris (CRC N 97010).

Program Nr: 953 from the 1999 ASHG Annual Meeting

Expression of *JAGGED1* gene in human embryos. *C. Crosnier[1], T. Attie-Bitach[2], S. Audollent[2], F. Encha-Razavi[2], F. Soudy[1], M. Hadchouel[1,3], M. Meunier-Rotival[1], M. Vekemans[2]*. 1) INSERM U347, Kremlin-Bicetre, France; 2) INSERM U393, Dept de Genetique, Hopital Necker, Paris, France; 3) Hopital de Bicetre, France.

Mutations of the *JAGGED1* gene encoding a NOTCH receptor ligand, cause Alagille syndrome (AGS), a malformative disorder affecting mainly liver, heart, vertebrae, eye, face. To study its role during human development and in the AGS phenotype, we performed in situ hybridization on paraffin-embedded 24- to 56-day old human embryos and on fetal tissues (liver and kidney at 11, 13 and 20 weeks, brain and heart at 20 weeks of gestation).

We showed that *JAGGED1* was expressed in the developing central nervous system (CNS), eye, ear, kidney, heart and blood vessels. In the CNS, it was strongly expressed in 2 stripes in the ventral neuroepithelium of the mesencephalon and rhombencephalon, and on either side of the roof plate. In the developing ear, *JAGGED1* was first seen in the ventral half of the otic vesicle, then in the developing sensory epithelium of the endolymphatic canal and the cochlea. In the eye, it was first detected in the lens placode, then in the lens and was finally restricted to posterior epithelial cells. A strong signal was observed in the ciliary body, hyaloid plexus and choroid. *JAGGED1* was strongly expressed early in the foregut mesenchyme, and later in the stomach and duodenum. In the kidney, it was first detected in the wolffian duct and mesonephric tubules, then in metanephric tubules. Finally, it was weakly expressed in the developing atria, but strongly in the conotruncus. *JAGGED1* was observed in all the developing vessels including the hepatic arteries of the liver at 20 weeks.

These observations are consistent with the features observed in various tissues in AGS patients, including minor features of the syndrome such as renal and forelimb abnormalities. Blood vessels were the structures which were most frequently labelled in human embryos, suggesting that abnormal angiogenesis/vasculogenesis might be involved in the pathogenesis of AGS features, including the paucity of interlobular bile ducts.

Program Nr: 954 from the 1999 ASHG Annual Meeting

Maternal smoking: age distribution, analyte levels and effect on detection of Downs syndrome pregnancies in second trimester screening. *J.A. Crossley[1], D.A. Aitken[1], S. Waugh[1], T. Kelly[2], J.M. Connor[1]*. 1) Biochemical Genetics Div, Inst Medical Genetics, Glasgow, Scotland; 2) Glasgow Royal Maternity Hospital, Glasgow, Scotland.

Information on maternal smoking was obtained by case-note searches in 101 Down's syndrome pregnancies and 2272 normal pregnancies which had been screened in the second trimester using AFP and hCG. In the affected pregnancies there were 75 non-smokers, 24 smokers and 2 who had recently stopped. In the unaffected pregnancies there were 1514 non-smokers, 671 (30%) smokers and 87 recently stopped. The median ages for the non-smokers and smokers were, respectively, in the Down's syndrome group 33 years and 31 years, and in the unaffected pregnancies 28 years and 25 years. The proportion of pregnant women who smoked decreased with increasing maternal age, from 52% in those aged under 20 years to 19% in those 35 years and over. Using maternal age risks in individual years the expected rate of Down's syndrome at term was calculated from the age distribution of smokers and non-smokers and found to be 1.1/1000 in the smokers and 1.4/1000 in the non-smokers. In the unaffected singleton pregnancies the median MOM (multiple of the median) for AFP was 1.00 overall: 0.98 in non-smokers and 1.04 in smokers, and for hCG, 1.00 overall: 1.13 in non-smokers and 0.80 in smokers. This reduction in hCG levels in smokers did not vary with the number of cigarettes smoked per day. In the Down's syndrome pregnancies the median MOM for AFP was 0.69 overall: 0.69 in the non-smokers and 0.70 in the current smokers, and for hCG 2.13 overall: 2.49 in the non-smokers and 1.53 in current smokers. The detection rate for Down's syndrome was 67% overall: 76% in the non-smokers and 38% in the smokers. Correcting the analyte levels for smoking status makes little difference to the overall detection rate (68%), but gives a more equal distribution of detection with 69% in the non-smokers and 63% in the smokers. Use of uncorrected medians therefore leads to loss of detection of Down's syndrome cases amongst smokers and an increase in the false positive rate amongst non-smokers.

Program Nr: 955 from the 1999 ASHG Annual Meeting

Early prenatal diagnosis of XLR centronuclear myopathy using mutation analysis and early histopathological changes in an affected fetus. *D. Cushing[1], S. Liechti-Gallati[2], A. Chan[1], V. Jay[3], D. Chitayat[1]*. 1) Prenatal Diagnosis Program, University of Toronto, Toronto, Ontario, Canada; 2) Department of Human Molecular Genetics, Children's Hospital, Inselspital, Bern, Switzerland; 3) Department of Pathology, University of Toronto, Toronto, Ontario, Canada.

X-linked centronuclear myopathy (XLR-CNM, OMIM #310400) is a myopathy resulting in severe hypotonia and respiratory failure. The disease is caused by a mutation in the MTM1 gene mapped to Xq28. We report early prenatal diagnosis in a family with XLR-CNM due to a 340insA mutation in exon 5 of the MTM1 gene and the fetal histopathological findings at 14 weeks gestation. The patient was a 34 year old woman whose first son was delivered at 28 weeks gestation with hypotonia and respiratory difficulties and died at 6 months of age. Muscle biopsy was consistent with CNM. Muscle biopsy on the patient demonstrated muscle fibres with increased central nuclei consistent with a carrier state for XLR-CNM. In her subsequent pregnancy the patient decided to pursue early prenatal diagnosis. Mutation analysis on the patient demonstrated she is a carrier of the 340insA mutation in exon 5 of the MTM1 gene(previously reported in XLR-CNM) and analysis of fetal DNA extracted from CVS showed the fetus to be an affected male. The pregnancy was terminated at 15 weeks gestation and histopathology confirmed prominent central nuclei and type 1 predominance consistent with CNM. Review of the family history showed that the patient had a brother and two maternal uncles who died in infancy of unknown etiology. To the best of our knowledge this is the first reported case of prenatal diagnosis of XLR-CNM by mutation analysis and the earliest fetal muscle histopathology showing that the muscle changes present very early in pregnancy. Since similar histological changes can be detected in normal fetuses, prenatal diagnosis is most reliable by mutation analysis.

Program Nr: 956 from the 1999 ASHG Annual Meeting

Two dimensional ultrasound detection of coronal synostosis at 19 weeks gestation leading to the prenatal diagnosis of Apert syndrome. *D. Day-Salvatore[1], E. Guzman[2], T. Cimaroli[1], J. Pellegrino[1], F. Schaefer[3], A. Vintzileos[2]*. 1) Division of Clinical Genetics, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ; 2) Division of Maternal-Fetal Medicine, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ; 3) H.A. Chapman Institute of Medical Genetics, Tulsa, OK.

The prenatal ultrasound detection of sporadic Apert syndrome has been previously described in eight cases. In these cases, presenting sonographic findings included ventriculomegaly, cloverleaf or other bizarre skull shape, abnormal facial profile with frontal bossing, and polyhydramnios; the diagnosis was ultimately made between 26 and 34 weeks of gestation. We report on a healthy 32-year-old prima gravida referred at 18 weeks 6 days gestation for strawberry-shaped cranium to rule out trisomy 18. Fetal ultrasound biometry was consistent with gestational age based on intrauterine insemination. Axial views of the cranium revealed frontal-parietal notching with fusion of the coronal sutures inferiorly. The superior aspect of the coronal sutures and the metopic, sagittal, and lambdoidal sutures at all levels were noted to be echolucent and patent. Ultrasound examination of healthy fetuses of similar gestational age demonstrated sutural patency at all levels. The fetal profile, intracranial anatomy, and amniotic fluid index in this case were normal. The hands appeared clenched bilaterally with no visible separation of the digits and both feet were abnormally shaped. Amniocentesis revealed a 46,XX karyotype. Molecular analysis of cultured amniocytes demonstrated an alteration of the FGFR2 gene in exon 7 at codon 253 leading to a proline-to-arginine substitution, one of two mutations previously described in Apert syndrome. Autopsy revealed complete syndactyly of all fingers and toes, partial coronal synostosis, and unremarkable facial appearance. This case supports the utility of standard prenatal sonography in identifying fetuses with coronal suture synostosis prior to the third trimester and the development of secondary craniofacial abnormalities.

Program Nr: 957 from the 1999 ASHG Annual Meeting

A report of the diagnostic and pronostic accuracy of prenatal sonographic diagnosis of skeletal dysplasias. *B. Doray[1], R. Favre[2], B. Viville[2], B. Langer[2], C. Stoll[2].* 1) INSERM U393, Inst Necker/IFREM, Paris, France; 2) Service de Genetique Medicale, Hopital de Hautepierre, Strasbourg, France.

As ultrasonographic assessment of the skeleton is now routine part of fetal anomaly scanning, the objective of this study was to examine the accuracy of prenatal diagnosis of skeletal dysplasias and ways to refine this ability. We report a series of 47 cases seen during a 6-year interval in which a skeletal dysplasia was suspected prenatally. Diagnosis was performed between 16 and 24 weeks of gestational age in 28 cases (59.6 per cent). Follow up was incomplete in two cases. In 45 (96.0 per cent) of the 47 cases, prenatal sonographic examination correctly predicted the prognosis, although in only 29 (67.7 per cent) of the 47 cases was the suggested prenatal diagnosis proven to be correct. In 9 cases (19.1 per cent) the prenatal diagnosis was inaccurate and in 10 cases (21.3 per cent) the prenatal diagnosis was false. In ten (21.3 per cent) of the 47 cases, a specific diagnosis could not to be made either pre-or postnatally. The most common final diagnosis were achondroplasia (7 cases), osteogenesis imperfecta (13 cases), thanatrophoric dwarfism (4 cases), achondrogenesis (5 cases) and Ellis van Creveld syndrom (3 cases). The diagnosis of achondroplasia was never made before 24 weeks of gestational age. In two cases (5.5 per cent) the fetus did not appear to have a skeletal dysplasia or an obvious dysmorphic syndrom. Termination of pregnancy was performed in 36 cases (76.6 per cent). Our study showed the difficulty of making an accurate prenatal sonographic diagnosis in fetuses with suspected skeletal dysplasias throughout gestation, especially in the third trimester, and the importance of comprehensive multidisciplinary assessment in these cases.

Program Nr: 958 from the 1999 ASHG Annual Meeting

A Spinal Muscular Atrophy Conundrum. *D. du Sart[1], I. Biros[1], S. Forrest[2], A. Bankier[1], M. Smith[1]*. 1) Victorian Clinical Genetics Services , Murdoch Institute, Melbourne, Victoria, Australia; 2) Gene Discovery Group, Murdoch Institute, Melbourne, Victoria, Australia.

Spinal muscular atrophy (SMA) is a common autosomal recessive disease affecting the motor neurones of the spinal cord. The critical SMA region on chromosome 5q13 shows a complex genomic structure, including a 500-kb duplication and inversion encompassing four genes, which are present in at least two copies each, the telomeric (t) and centromeric (c) counterparts. Homozygous deletions of the telomeric copies of these genes are found in SMA patients, with the survival motor neuron (SMN) gene showing the highest frequency, deleted in 95% of SMA patients. The diagnostic test for SMA linked to chromosome 5 has been analysis for homozygous deletion of the SMNt. Prenatal testing for SMA involves the direct deletion gene test of SMN and linkage analysis with polymorphic markers close to the critical SMA region. There have been suggestions that the linkage analysis is not necessary for a prenatal exclusion if a previously affected sibling was genotyped to be homozygously deleted for SMNt. We tested a CVS sample using the deletion test and JK53 and EF1/2a linkage markers. The previously affected sibling was homozygously deleted for SMNt. The CVS was found to have at least one copy of SMNt and the semi-informative linkage result indicated that the CVS sample was at least a carrier of SMA. The pregnancy was continued. The child was born and started displaying symptoms of SMA at 6 weeks. SMA gene testing was repeated and the second result showed homozygous deletion for SMNt. Maternal cell contamination was ruled out. The SMA gene test was repeated on all samples, including 10 different placental tissue biopsies (the parents kept the placenta for personal reasons). All placental tissue samples confirmed the CVS results. New closer linkage markers, D5S1414 and D5S1408, indicated that the CVS and placental samples had inherited the high-risk chromosomes from both parents and therefore should have been homozygously deleted for SMNt. How do we explain this conundrum? We therefore propose, that prenatal diagnosis should include both the direct deletion gene test and linkage analysis.

Program Nr: 959 from the 1999 ASHG Annual Meeting

PRENATALLY DIAGNOSED BALANCED ROBERTSONIAN OR RECIPROCAL TRANSLOCATIONS AND FREQUENCY OF UPD. *J.R. Exeler[1], D. Meschede[1], M. Lemmens[2], B. Dworniczak[1], J. Horst[1]*. 1) Universitaet Muenster, Humangenetik, D-48149 Muenster, NRW, Germany; 2) Humangenetik, Eisenhuette 23, D-52076 Aachen, NRW, Germany.

The term uniparental disomy was originally used by Engel in 1980 and implies the inheritance of both homologous chromosomes from one parent. UPD may adversely affect development if homozygosity for recessive gene mutations occurs or imprinting effects are present. An increased risk for UPD has been identified in several groups including carriers of mosaic trisomies, fetuses after prenatal diagnosis of confined placental mosaicism, and carriers of balanced Robertsonian or reciprocal translocations. In this study we investigated the frequency of UPD after the incidental prenatal diagnosis of a balanced Robertsonian translocation in a total of 25 cases and a balanced reciprocal translocation in 10 cases, respectively. In all cases the fetal translocation had originated from an analogous maternal or paternal translocation. To establish the parental origin of the involved chromosomes highly polymorphic dinucleotide repeats were analysed. Informative results were obtained without exception. UPD of the affected chromosomes could be excluded in all 35 cases. We conclude that UPD has a low prevalence among carriers of balanced Robertsonian or reciprocal translocations who are diagnosed incidentally in the setting of prenatal diagnosis.

Program Nr: 960 from the 1999 ASHG Annual Meeting

Chromosome 8p rearrangements are a cause of prenatal conotruncal cardiopathies. *P.L. Eydoux[1], O. Dupuy[1], J. Wirth[2], N. Collot[1], A. Azancot[3], C. Baumann[3], S. Magnier[4], E. Vuillard[3], J.F. Oury[3], P. Blot[3], C. Nessmann[1]*. 1) Lab De Biol du Development, Hosp Robert-Debre, Paris, France; 2) Max-Planck Institute for Molecular Genetics, Berlin, Germany; 3) Obstetrics-Gynecology, Hosp Robert-Debre, Paris, France; 4) Cardiology, Hosp Robert-Debre, Paris, France.

Conotruncal malformations are a frequent clinical feature of chromosome 22q11.2 microdeletion and other anomalies such as chromosome 10p13 microdeletion. Chromosome 8p rearrangements have also been reported as a possible cause of these cardiopathies. We report two patients with a conotruncal malformation detected through ultrasound examination, and a rearrangement of chromosome 8p. In one case, an amniocentesis was performed because of a thickened nuchal fold at 15 weeks of gestation. The karyotype was normal, and an echocardiography showed a regular form of tetralogy of Fallot. No microdeletion of chromosome 22q11.2 was detected. After birth, the cardiac malformation was confirmed, and developmental delay and a cranio-facial dysmorphism were noted. Blood karyotyping showed an excess of the short arm of chromosome 8; whole chromosome painting indicated an intrachromosomal rearrangement. Parental karyotypes were normal. Using YAC probes, a deletion was detected in band 8q23. Clinical and cytogenetic findings are thus compatible with the recombinant chromosome 8 syndrome. In the other patient, intra-uterine growth retardation and a cardiac malformation were detected at 22 weeks. Prenatal echocardiographic diagnosis was a double outlet right ventricle. Amniotic fluid cells karyotyping showed an excess of the short arm of chromosome 8; parental karyotypes revealed a translocation in the mother (46,XX,t(2;8)(q34;p22)). Thus, the fetus had the der(8) of maternal origin, with partial trisomy of chromosome 2q and partial monosomy of chromosome 8p. Termination of pregnancy was denied by the parents, and the pregnancy is still ongoing. Chromosome 8p deletions should be emphasized as a possible cause for conotruncal cardiac malformations. Molecular cytogenetic studies will be useful for prenatal diagnosis of this condition, and for delineation of the critical region of chromosome 8 involved in conotruncal malformations.

Program Nr: 961 from the 1999 ASHG Annual Meeting

Familial Lethal X-linked Arthrogryposis Syndrome in an Extended Mexican Kindred: Results from Fetal Ultrasound, Autopsy and Molecular Investigations. *J.C. Ferreira[1], P. Dar[1], N. Tenney[2], S.M. Carter[1], L.L. Baumbach Reardon[3], S.J. Gross[1]*. 1) Montefiore Med Ctr, Bronx, NY; 2) Woodside FHC, Woodside, NY; 3) Univ Miami School Med, Miami, FL.

Background: Pena-Shokeir syndrome is a lethal arthrogryposis syndrome that seems to be secondary to a motor neuron disorder (MIM 208150) or to developmental brain anomalies (MIM 214150). Inheritance is consistent with autosomal recessive transmission. However, some cases suggesting X-linked transmission have also been described, most have been related to a motor neuron disorder (MIM 301830) with possible linkage to Xp11-Xq11(Hum Mol Genet.,4(7):1213-6,1995). Additionally, a few families have been reported in which only males were affected with an arthrogryposis secondary to brain anomalies, suggesting yet another X-linked syndrome(MIM 300073). We describe a family with males presenting with a lethal arthrogryposis syndrome, secondary to brain anomalies. X-linked transmission is strongly suggested.

Case:: The proband, a 27 yo, G6P4 of Mexican descent had two sons who died perinataly. Both were affected with an arthrogryposis syndrome and brain anomalies. Family history is significant for two stillborn siblings, one with clubbed feet. In the current pregnancy the fetus,46XY, was found sonographically at 19 weeks to be growth restricted with clubbed feet, clenched hands and cerebellar hypoplasia. Based on these findings, the pregnancy was terminated. Fetal autopsy revealed severe growth restriction and multiple digit and limb contractures. The spinal cord was normal but adequate evaluation of the brain was not possible. Blood samples from the entire family are being collected and being used to examine potential linkage to Xp11.1-3 region referenced above.

Conclusion: This family reinforces the hypothesis that X-linked form of arthrogryposis exists. The possible allelic nature of this syndrome with previously reported cases is being examined, and our findings will be reported.

Program Nr: 962 from the 1999 ASHG Annual Meeting

Multiple congenital anomalies in a fetus with male and female cell lines: a chimera? *K. Filkins[1], N. Rao[2], W. Robinson[3], L. Chen[1], T. Markuson[1], S. Poggi[1], F. Kaselonis[4], S. Cederbaum[4].* 1) Dept OB/GYN, Univ California, Los Angeles, Los Angeles, CA; 2) Dept Pathology, Univ California, Los Angeles, CA; 3) B.C. Research Institute for Children's & Women's Health, Univ British Columbia, Vancouver, BC, Canada; 4) Dept Pediatrics, Univ California, Los Angeles, CA.

Prenatal expanded MSAFP screening done in a 31 year old G1 P1 A6 yielded a screen positive result for Trisomy 18. The patient was referred for genetic counseling but she declined amniocentesis because she would not consider termination of the pregnancy even for a lethal condition. Ultrasound evaluation at 22 weeks revealed growth consistent with dates except for a week lag of the abdominal circumference. Severe oligohydramnios along with limb posturing (possibly secondary to the oligohydramnios) was noted. The nuchal fold was increased to 7-10 mm. The fetal stomach and bladder were eventually visualized but the cardiac and facial evaluations were hampered due to lack of fluid. At 28 weeks gestation the abdominal circumference lagged 3 weeks and the femur length 4 weeks.

Chromosome analysis revealed two cell lines (approximately 50:50): 46,XY, der(16) 16ps and 46,XX. It was initially thought that the 46,XX line might represent maternal cell contamination, but the der(16)line was of maternal original and was not in the female cell line. Therefore, the likelihood of a chimeric 46,XY/46,XX could not be ruled out. At 38 weeks gestation a 2000 gm fetus was delivered with ambiguous genitalia,very small eyes with down slanting palpebral fissures, short sternum and profound limb abnormalities. The infant expired within 24 hrs. Further studies are pending to determine parental origins of the two cell lines and these will be reported along with the possible mechanisms for this abnormality.

Program Nr: 963 from the 1999 ASHG Annual Meeting

An assembly mutation in cartilage oligomeric matrix protein causes delay in skeletal development. *K.G. Gaiser*[1,2], *W.A. Horton*[1,2]. 1) Research Unit, Shriner's Hosp for Children, Portland, OR; 2) Dept. of Molecular and Medical Genetics, OHSU, Portland, OR.

Cartilage oligomeric matrix protein (COMP) is a pentameric, non-collagenous glycoprotein found abundantly in cartilage, tendon, ligament and synovium. Each COMP pentamer is composed of five identical monomers, which are assembled by a coiled-coil domain. Mutations within the COMP gene have been found to cause two types of dominantly inherited chondrodysplasias, pseudoachondroplasia (PSACH) and multiple epiphyseal dysplasia (MED). These disorders are characterized by mild to severe short stature and early onset osteoarthritis. Cartilage samples of PSACH and MED patients show an accumulation of material in the chondrocyte rough endoplasmic reticulum (RER). The effects of COMP mutations in these patients suggest that COMP function is essential for normal bone growth. However, there is a limited understanding of how COMP is assembled, its functional role(s) and how it is involved in bone development. In an attempt to understand the function of COMP, transgenic mice have been generated, which have a mutation in COMP assembly. By overexpressing a truncated monomer containing the coiled-coil domain, a dominant negative mutation has been created. The transgenic F1 offspring appear slightly smaller in size compared to the wild-type. Affected mice seem to be less active, but otherwise healthy and fertile. Further studies to characterize this phenotype are underway.

Program Nr: 964 from the 1999 ASHG Annual Meeting

Assessment of the genetic influence on human sperm morphology: A twin study. *N. Gursoy[1], J. Winters[1, 3], C. Clark[2], H. Maes[1], C. Jackson-Cook[1]*. 1) Dept. of Human Genetics, Virginia Commonwealth University, Richmond, VA; 2) Department of OB/GYN, Virginia Commonwealth University; 3) Case Western University and University Hospitals of Cleveland, Ohio.

Spermatogenesis is a very complex process involving multiple mitotic divisions, genetic recombination, two meiotic divisions and extreme morphologic changes. This unique process of differentiation is believed to be under genetic regulation by "unknown" genes. To assess the genetic influence on human sperm morphology and its possible association with sperm aneuploidy levels we evaluated sperm morphology and aneuploidy frequencies in 23 monozygotic (MZ) and 5 dizygotic (DZ) twin pairs (a total of 56 males). The ages of the donors ranged from 26 to 59 years. The zygosity of the twin pairs was confirmed using a panel of six highly polymorphic markers. Morphology of spermatozoa was assessed according to WHO guidelines. Aneuploidy levels for chromosomes X, Y, 3, 7, 8, 9, 15, 17, and 18 were determined using tri-color FISH. The analyses were performed blindly. There was no significant correlation between the proportion of morphologically abnormal spermatozoa and the proportion of aneuploidy in a male. However, a very significant correlation was found between genetically identical MZ co-twins for sperm morphology ($r = 0.931$). To determine the variation between two ejaculates within a male, sperm morphology analyses were repeated in a subset of 28 individuals (with > a 2 months interval). No significant differences in sperm morphology were noted between the first and second ejaculates in these men. To quantify the contribution of genetic and/or environmental factors to morphology, model fitting was performed using the structural equation modeling program Mx. This path analysis showed highly significant family resemblance for sperm morphology, most likely due to additive genetic factors. The very significant correlation between genetically identical twins as well as the results of path analysis suggests a strong genetic influence on sperm morphology. Studies of additional twins will allow us to better quantify the contribution of the genetic and/or shared environmental factors influencing sperm morphology.

Program Nr: 965 from the 1999 ASHG Annual Meeting

The Effect of Gravidity and Maternal Age on The First Trimester Down Syndrome Biochemical Markers Free-b **hCG and PAPP-A.** *T.W. Hallahan[1], D.A. Krantz[1], P.D. Buchanan[2], J.W. Larsen, Jr.[3], J.N. Macri[1].* 1) NTD Laboratories, Huntington Stat, NY; 2) GeneCare Medical Genetics Center, Chapel Hill, N.C; 3) The George Washington Univ. Medical Center, Washington, D.C.

Historically, maternal factors such as weight, ethnicity and diabetic status have been used to adjust second-trimester biochemical markers to refine the screening process and improve false positive and detection rates. The effects of maternal age and gravidity on second-trimester biochemical markers are small and generally do not need to be taken into account. To determine the effect of gravidity and maternal age on first-trimester biochemical markers, dried blood specimens from 1884 patients between 8w2d and 13w6d gestation were analyzed for free-b hCG and PAPP-A. The table shows median weight adjusted MoM levels of free-b hCG and PAPP-A vs. gravida and maternal age.

	Gravida				Age		
	1	2	3	>=4	<=30	30-34	>=35
free-b	0.97	0.99	1.08	1.04	0.95	1.00	1.04
PAPP-A	0.99	0.99	1.02	1.03	0.95	0.99	1.07

The rank correlation coefficient of free-b with gravidity and age was 0.05 and 0.04 respectively, while for PAPP-A they were 0.04 and 0.10, respectively. Only the correlation of PAPP-A and age was statistically significant (P<.001), however this association was quite small, especially in light of the association of gravida and age. In conclusion, although maternal age plays a significant role in Down syndrome screening as a major factor in determining prior risk, maternal age and gravidity have little or no effect on the serum levels of first trimester biochemical Down syndrome screening markers.

Program Nr: 966 from the 1999 ASHG Annual Meeting

High level *SOX9* expression during development of the human embryonic pancreas is associated with abnormal islet formation in a case of Campomelic Dyplasia. *N.A. Hanley[1], K. Piper[1], S.G. Ball[2], M. Clement-Jones[1], T. Strachan[1], J.W. Keeling[3], D.I. Wilson[1,2].* 1) School of Biochemistry & Genetics; 2) Dept. of Medicine, University of Newcastle, Newcastle upon Tyne, UK; 3) Department of Pathology, Royal Hospital for Sick Children, Edinburgh, UK.

SOX9 is a high mobility group (HMG) domain transcription factor that is important for chondrogenesis and testicular determination. Human mutations in the *SOX9* gene result in the haploinsufficiency disorder, Campomelic Dysplasia, characterised by severe skeletal malformations and male-to-female sex reversal. Although not uniformly fatal *in utero*, death due to respiratory problems almost always occurs prior to 5 years.

The pancreas develops as ventral and dorsal outgrowths of the foregut endoderm. During human embryogenesis, we show that *SOX9* is expressed from the inception of pancreas development (26 days post-conception) at levels comparable to the developing skeleton and testis. This expression throughout the entire organ decreases during the second trimester and, in particular, is seemingly extinguished within cells that have differentiated towards an endocrine lineage.

Analysis of the pancreas from a neonatal case of classical Campomelic Dysplasia shows that gross morphology is intact, however, despite the presence of all major cell types, immunocytochemistry reveals an abnormal histological appearance with disordered islet structure. In the absence of glucose intolerance, the insulin-glucagon core of islets appears smaller than those of normal controls, with a preponderance of outlying cells positive for pancreatic polypeptide.

The pancreatic phenotype of this neonate and the high level of pancreatic *SOX9* expression during normal human embryogenesis suggest a hitherto unrealised and potentially important role for this transcription factor during human pancreas development. High mortality during early life makes it difficult to interpret a clinical lack of glucose intolerance. A role for *SOX9* in determining beta cell mass, in addition to islet formation, therefore cannot be excluded and warrants further investigation.

Program Nr: 967 from the 1999 ASHG Annual Meeting

Prenatal diagnosis of cutis laxa by fetal echocardiography. *P. Himes[1], L. Linck[2], P. Silberberg[3], M.J. Rice[4], L. Hankenson[5]*. 1) Dept Mol & Medical Genetics, Oregon Health Sci Univ, Portland, OR; 2) Kaiser Permanente, Clackamas, OR; 3) Dept Radiology, Oregon Health Sciences Univ, Portland, OR; 4) Dept Cardiology, Oregon Health Sciences Univ, Portland, OR; 5) Portland, OR.

Cutis laxa is a heterogeneous condition associated with lax skin and, frequently, systemic abnormalities involving the lungs, arteries and urinary tract. Cutis laxa has been associated with lysyl oxidase deficiency and elastin mutations. Autosomal dominant, autosomal recessive and X-linked recessive inheritance have been reported, but it may also be an acquired condition. When present in the newborn period, the condition is usually due to autosomal recessive inheritance. In the absence of a positive family history or a known mutation in the elastin gene, definitive prenatal diagnosis may not be possible. We report a case of prenatal diagnosis of cutis laxa by fetal echocardiography at 23 weeks gestation in a pregnancy known to be at risk. The couple's first child had been diagnosed with cutis laxa at birth. Associated findings included an atrial septal defect and pulmonary artery stenosis, a markedly tortuous aorta, diverticuli of the esophagus and bladder, bilateral inguinal hernias, and bronchomalacia. Laboratory studies included normal serum copper and ceruloplasmin levels; a urine pyridinoline assay of collagen cross-links showed a marked increase in pyridinoline relative to deoxy-pyridinoline. She died when seven months old. In the subsequent pregnancy, fetal echocardiography was performed at 23 weeks' gestation which showed an atrial septal aneurysm, mild tricuspid regurgitation and a tortuous aortic arch. Lax skin with excessive folds was noted at birth and the child was diagnosed with cutis laxa. Elevated right ventricular pressure, tricuspid insufficiency, branch pulmonary stenosis and tracheobronchomalacia were present. Interestingly, a urine pyridinoline assay was normal. He developed severe pulmonary emphysema and died when three months old. This case demonstrates the usefulness of fetal echocardiography for prenatal diagnosis of some cases of cutis laxa.

Program Nr: 968 from the 1999 ASHG Annual Meeting

Expression of the Rieger syndrome Pitx2 protein in mouse development. *T. Hjalt[1], E.S. Semina[1], B.A. Amendt[2], J.C. Murray[1]*. 1) Dept Pediatrics, Univ Iowa, Iowa City, IA; 2) Dept Physiology, Univ Iowa, Iowa City, IA.

The Rieger syndrome, an autosomal dominant disorder involving ocular, dental, and umbilical defects is caused by mutations in PITX2, a Bicoid type homeobox protein. Moreover, Pitx2 is involved in the Nodal/Sonic hedgehog pathway that determines left/right polarity. Mouse Pitx2 mRNA is expressed in eye, tooth and umbilicus consistent with the human Riegers phenotype. It is also asymmetrically expressed on the left side in the early lateral plate mesoderm, and later to the left in structures of the gut and the heart. In this report we demonstrate a 32 kDa polypeptide on western blots of nuclear extracts from a rat pituitary cell line, using a Pitx2 specific antibody (designated P2R10). The rabbit anti human P2R10 was specific for a region in the N-terminus, preceding the homeodomain. The epitope is conserved between human, mouse, and Xenopus Pitx2. We describe also expression of the Pitx2 protein in mouse. Pitx2 protein immunostaining was detectable during the development of the eye, tooth, umbilicus, and also in the pituitary, heart, gut, and limb. In the developing eye, Pitx2 protein was expressed in the corneal ectoderm, and in late stages in the iris. We detected asymmetric expression of Pitx2 protein in the early heart and gut. Pitx2 was also expressed in the septum primum of the heart. We further show nuclear localization for transiently transfected PITX2, as well as for endogenously expressed PITX2 in a pituitary cell line. The Rieger syndrome mutation T68P was also shown to be nuclear localized. These localizations are consistent with the Rieger phenotype and support a role for PITX2 in cardiac development as well.

Program Nr: 969 from the 1999 ASHG Annual Meeting

Prenatal Diagnosis of Desbuquois Syndrome. *W.A. Hogge[1,2], T. Prosen[1,2], E. McPherson[1], L. Hill[2], K. Lain[2].* 1) Dept Genetics, Magee Womens Hosp, Pittsburgh, PA; 2) Dept OB/GYN, Magee Womens Hosp, Pittsburgh, PA.

We present a case of Desbuquois Syndrome diagnosed prenatally in a family without a prior history of the disorder. A 29 year old Middle Eastern G2 P1 was referred for evaluation after a routine dating ultrasound at 21 weeks was suspicious for skeletal dysplasia. Long bones measured <5%ile. The ultrasound was also notable for bilateral clubbed feet. Family history was unremarkable without a history of skeletal dysplasia. Consanguinity was denied. A detailed ultrasound at our institution revealed shortening of all long bones (<5%ile) as well as shortening of the fetal foot. The ribs measured at the 50%ile for gestational age and the chest circumference was at the 10th to 25th percentile. Bilateral syndactyly of the third, fourth, and fifth digits of the hands was suspected. The hands also had a significant separation of the second and third digits. Bilateral clubbed feet with a rocker bottom appearance were noted. The right foot was thought to have polydactyly and the toes of both feet appeared unusually long, except for the great toes which appeared shortened. The fetal eyes protruded from the orbits. Fetal echo was normal. Karyotype was 46,XX. Cytogenetic studies for Robert's Syndrome were negative. A repeat ultrasound 13 days later had additional findings of polydactyly of both feet. The couple opted to terminate the pregnancy and underwent labor induction at 23 weeks gestation. Autopsy findings included short long bones of both the upper and lower extremities; short, stubby, tapered fingers; clinodactyly of the 5th fingers bilaterally; short, stubby, great toes bilaterally with wide space between 1st and 2nd toes; abnormal palmar creases; short, broad neck; micrognathia & hypoplastic lungs. X-rays revealed supernumerary ossification center between the proximal phalanx and second metacarpal of the index finger. Conclusion: We suspected the diagnosis based on the extreme shortening of the long bones coupled with the abnormal positioning of the fetal hands. The polydactyly, in retrospect, likely reflected the presence of supernumerary ossification centers. These may be clues to the prenatal diagnosis of this rare autosomal recessive skeletal dysplasia.

Program Nr: 970 from the 1999 ASHG Annual Meeting

Are there better antibodies than CD71 for the detection of fetal nucleated cells out of maternal blood? *H. Hohmann[1], S. Michel[1], W. Reiber[2], M. Günther[3], T. Graf[4], U. Claussen[1], F. von Eggeling[1]*. 1) Institute of Human Genetics and Anthropology, University of Jena, Jena, Germany; 2) Private Gynecologist, Jena, Germany; 3) Department of Obstetrics, University of Jena, Germany; 4) Albert Einstein College of Medicine, New York, USA.

The availability of fetal cells from the maternal peripheral blood opens up the possibility for a non-invasive prenatal diagnosis. Up to now this prospect is hampered by the fact that there are no highly fetal specific antibodies. Various studies have been done concerning the identification of specific cell surface antigen markers to facilitate the specific isolation of fetal nucleated erythrocytes (e. g. CD71, glycophorin A, CD36). In the present study four up to now untested monoclonal antibodies 2G5, 3F9, EPO-R and Flk1 were analysed in view of their effectiveness to stain fetal nucleated cells. 2G5 and 3F9 are avian cell specific antibodies, which seem to be suitable in human because they can differentiate between fetal and adult nucleated erythrocytes. EPO-R is a monoclonal antibody against the erythropoeitin receptor. Flk1 is a monoclonal antibody against the fetal liver kinase 1 and is required for the embryonic development of vascular endothelial and hematopoietic cells. As reference the commonly used antibody against the transferrin receptor CD71 was constituted. 20 ml of peripheral blood from 40 women carrying a male fetus were collected in the 16th to 28th week of pregnancy. Mononucleated cells, enriched by density gradient, were stained with one of the four antibodies and isolated with magnetic activated cell sorting (MACS). Succeeding fluorescence in situ hybridisation (FISH) with chromosome X and Y centromere probes allowed to analyse the origin of the cells. For evaluation 500 nuclei per case (n) were examined. The antibody 3F9 detected 0.2 cells on average (n=5), 2G5 1.2 (n=11), EPO-R 1.6 (n=10) and Flk1 1.9 cells (n=8) on average with fetal specific Y-positive hybridisation signals. The antibody against CD71 (n=6) used as reference gave no Y-specific signals. Considering these results the tested antibodies, especially Flk1 seem to be more effective in detecting fetal cells than the CD71 antibody.

Program Nr: 971 from the 1999 ASHG Annual Meeting

Use of fetal cells in maternal blood for FISH - and PCR - based prenatal diagnosis . *W. Holzgreve[1], X.Y. Zhong[1], C. Troeger[1], P. Miny[2], S. Tercanli[1], S. Hahn[1]*. 1) Dept. OB/GYN, University of Basel, Basel, Switzerland; 2) Dept. Human Genetics, University of Basel, Basel, Switzerland.

Aim: Our laboratory has pioneered the use of Magnetic Cell Separation (MACS) for the enrichment of fetal cells from the blood of pregnant women. As participants of the NICHD Fetal cell isolation study (NIFTY), we are currently testing the efficacy of using such cells for the prenatal diagnosis of fetal aneuploidies. We have further extended upon this study by examining single enriched fetal cells isolated by micromanipulation by single cell PCR for Mendelean disorders.

Methods: Fetal cells are enriched from peripheral blood samples (15 ml) obtained from pregnant women (12 - 16 weeks gestation) by the use of miniMacs columns and either anti-CD71 or anti-glycophorin A. Fetal cells are identified either by multicolor FISH or by single cell PCR for fetal specific loci such as rhesus D or the SRY gene.

Results: In an examination of 520 samples by XY FISH, we were able to correctly determine fetal cells in 57 percent of the cases. When two or more XY positive cells were used as being indicative of male fetal sex, specificities of over 95 percent could be attained. In an examination of 14 samples obtained from rhesus D negative women, both the fetal sex and rhesus D genotype were correctly determined in all instances by the use of multiplex PCR on single isolated fetal erythroblasts.

Conclusion: We have shown that fetal cells can be reliably enriched from maternal blood, and that they can be used for the detection of specific chromosomes by FISH and for the analysis of fetal genetic loci by single cell PCR with specificities superior to current non-invasive means.

Program Nr: 972 from the 1999 ASHG Annual Meeting

Successful pregnancy in a patient with severe deforming osteogenesis imperfecta (O.I.). *L. Hudon[1], G.N. Willey[2], V. Enciso[3], S. Longmire[2], I.B. Van den Veyver[1,3]*. 1) Dept of Obstetrics and Gynecology; 2) Dept of Anesthesiology; 3) Dept of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

There are few reports of successful pregnancy in severe deforming O.I. We describe here the pregnancy outcome of a wheelchair-bound patient with severe O.I. and extreme short stature, and make recommendations for a multidisciplinary approach in these cases. The proband was born with fractures and was diagnosed with severe O.I., likely type III. She sustained multiple fractures requiring surgical intervention throughout childhood. The patient presented at 12 weeks gestation. Her height and weight were 117 cm and 19 kg and she had severe scoliosis and long bone deformities. Prenatal diagnosis was declined. The following specific risks were anticipated: cardiopulmonary compromise with advancing gestation due to short stature and thoracic deformities; preterm labor and delivery; poor maternal and fetal weight gain and defective nutrition; increased anaesthetic risk at delivery. A baseline echocardiogram and pulmonary function tests were normal for her stature. Optimal nutrition resulted in a 15 kg weight gain. Weekly steroids for fetal lung maturation were started at 24 weeks. Delivery by cesarean section was planned. Based on the results of a CT scan with 3 dimensional reconstruction of the lumbar spine regional anesthesia was found to be technically impossible. Baseline and pre-operative chest X-ray, arterial blood gas and echocardiogram were obtained and were normal. The patient presented at 30 4/7 weeks gestation in active labor. Elective fiberoptic awake intubation was performed because of difficult airway passage. A healthy male infant weighing 1120 g with no apparent fractures was delivered by classical cesarean section. The patient was discharged after 3 days in good condition. The newborn was discharged with no apparent sequelae and no clinical and radiological evidence of OI. Adult female patients severely affected with genetic conditions often desire pregnancy. This report illustrates that this is possible despite significant risks if potential complications are anticipated and managed in a tertiary care center by a team approach.

Program Nr: 973 from the 1999 ASHG Annual Meeting

Prenatally diagnosed isolated Dandy-Walker variant associated with 4p- and 6p- karyotypes. *L. Hunnisett[1,5], A. Toi[1,2,5], E. Winsor[1,3,5], S. Blaser[4,5], D. Chitayat[1,5]*. 1) Prenatal Diagnosis Program, The University Health Network; 2) Department of Diagnostic Imaging, The University Health Network; 3) Department of Laboratory Medicine, University Health Network; 4) Department of Radiology, Hospital for Sick Children; 5) University of Toronto, Toronto, Ontario, Canada.

Dandy-Walker Variant (DWV) is a posterior fossa anomaly with partial agenesis of the cerebellar vermis. The etiology is heterogenous and the prognosis is difficult to predict. In the largest reported series (Estroff, et.al., 1992) an abnormal karyotype was found in 5/17; however, other sonographic abnormalities were detected in all of them. We report two fetuses, with isolated partial agenesis of the cerebellar vermis who were found to have abnormal karyotypes: 4p- and 6p-. Case 1: A 31-year-old G1P0 woman of Fillipino descent presented at 18.7 weeks gestation with query isolated DWV. Fetal brain MRI at 24 weeks gestation confirmed partial hypoplasia of the cerebellar vermis. Fetal karyotype was 46,XX,del(4)(p16.1). The pregnancy was terminated and vermian hypoplasia was confirmed at autopsy. In addition, autopsy revealed persistent left superior vena cava, abnormal thyroid gland and facial features consistent with Wolf-Hirschhorn syndrome. Parental karyotypes were normal. Case 2: A 26-year-old G1P0 woman of German descent presented at 18 weeks gestation with query isolated DWV. Subsequent ultrasound examinations confirmed isolated partial agenesis of the vermis and a small posterior fossa cyst. Fetal karyotype was 46,XY, del(6)(p25). The pregnancy was terminated and the autopsy report is pending. Parental karyotypes were normal. DWV is not a rare finding on fetal ultrasound, however not much is known about the etiology and prognosis. Although fetuses with isolated DWV usually have normal karyotypes and a good prognosis, our cases suggest that chromosome analysis should be considered even in the absence of other ultrasound abnormalities.

Program Nr: 974 from the 1999 ASHG Annual Meeting

Embryonic Alphafetoprotein Expression: Significant Human and Mouse Differences. *E.A. Jones[1,2], M. Clement-Jones[1], S. Lindsay[1], T. Strachan[1], S. Robson[1], D.I. Wilson[1,2].* 1) School of Biochemistry and Genetics, University of Newcastle upon Tyne, Newcastle upon Tyne, Tyne and Wear, UK; 2) School of Clinical Medical Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne, Tyne and Wear, UK.

PURPOSE : Alphafetoprotein (AFP) is a major serum protein in early fetal and embryonic life. It is initially produced by the yolk sac and later by hepatocytes. Expression is down regulated at birth but can be re-expressed in tumors such as hepatocellular carcinoma. However, despite extensive investigation, its function is still unknown. In order to define when and where AFP is produced during the embryonic period we have investigated the expression of AFP in mouse and human embryos.

METHODS : We have used human embryos (26-52 days post ovulation - dpo) from the Newcastle Human Embryo Collection and mouse embryos (9ed-13ed) for tissue in situ hybridisation using ^{35}S labelled riboprobes for AFP.

SUMMARY OF RESULTS : AFP is expressed in the hepatic diverticulum in mice and humans as it differentiates from the foregut endoderm and continues in hepatocytes throughout the embryonic period. AFP is also expressed in the yolk sac and gastrointestinal tract throughout the embryonic period. There is weak pancreatic expression in 41 dpo human embyos and 12ed mouse. However, in human embryos there were addditional sites of expression not observed in murine embryos. At 32 dpo the mesonephric duct expressed AFP and later the mesonephric tubules also expressed AFP. Expression was not seen in the human metanephros.

CONCLUSION : The major sites of AFP expression in man and mouse are hepatocytes, yolk sac, gastrointestinal tract and pancreas. Significantly, AFP is expressed in the mesonephric duct and tubules in humans but not in the mouse.

Program Nr: 975 from the 1999 ASHG Annual Meeting

Results of cytogenetic analysis of 560 amniotic fluid cell cultures (amniocenteses) performed in Iran. *M.H. Karimi-Nejad, A. Karimi-Nejad, N. Lashgarian.* Pathology & Genetics Ctr, Tehran, Iran.

From Nov. 1989 until 20 June 1999, more than 900 prenatal tests have been performed in this center for the following purposes: 598 cases for chromosomal abberation, 245 for Hemoglobinopathy, 41 for Inborn Errors of Metabolism, 11 cases for Tri- Nucleotide repeats, 7 for Muscular disorders, 5 for Skin lesions.

The indication and results of cytogenetic analysis of 560 amniotic cell cultures performed mostly at 13-16 wks of gestation are being reported. 546 (97.5%) fetuses were cytogenetically normal, or had balanced karyotypes. 14 (2.5%) had unbalanced karyotypes, and 2 fetuses were found to have major abnormalities, one with anencephaly detected by measurement of Alpha feto protein levels in amniotic fluid and ultrasonography, and the other with a full mutation at the FMR1 locus detected by molecular techniques. The unbalanced karyotypes include 2 cases each of trisomy 21, trisomy 18, Triple X, 47,XXY, and mosaicisms; and 4 cases of various chromosomal abnormalities.

245 tests were performed for women 35 years of age or over; 6(2.45%) abnormal karyotypes were detected, which shows a 6 fold increase over the general population. This risk was even much higher 2/27 (7.4%) for those parous aged>35 y with previous history of offspring with chromosomal aberrations.

5 of 34 (14.7%) fetuses whose parent was carrier of a balanced chromosomal translocation or a small chromosomal marker (in one case), had unbalanced chromosomal aberrations. 15 and 14 of the remainder revealed normal and balanced Karyotypes similar to their parents respectively.

There were 7 cases of spontaneous abortion within 4 wks after amniocentesis, one of which was a case of 46,XY,t(14;21),+18. Excluding this case, the rate of abortion 6/560 (1.07%) was quite below the expected rate of 2.1% for spontaneous abortion in the 2nd trimester of pregnancy. To the best of our knowledge the first chromosomal study of amniotic fluid cells in Iran was in November 1989, and at our center.

Program Nr: 976 from the 1999 ASHG Annual Meeting

Numerical abnormalities for chromosomes 1, 13, 16, 18, 21, 22, X and Y from 59 preimplantation embryos using mulit-color fluorescence *in situ* hybridization. *W.G. Kearns[1,2,3], M. Franzitta[1], S. Gitlin[2], M.W. Stacey[1], S. Phillips[2], W.E. Gibbons[2]*. 1) Center for Pediatric Research; 2) Jones Inst for Reproductive Med, EVMS, Norfolk, VA; 3) Inst. Med Genet. Johns Hopkins University School of Medicine, Baltimore, Maryland.

Objective: This study determined aneuploidy in blastomeres from 59 cleaving preimplantation embryos in which two pronuclei were observed 1 day after insemination. With IRB approval, embryos were donated by patients after informed consent was obtained. **Study Design:**Multi-probe, multi-color FISH was performed on blastomeres to diagnose aneuploidy for chromosomes 1,13,16,18,21,22,X and Y. Materials and **Methods:**Blastomeres from 6-8 cell preimplantation embryos were isolated. Individual blastomeres were dropped onto microscope slides and fixed. For all embryos, all available blastomeres were studied. First, simultaneous five-probe, four-color FISH was performed for chromosomes 13,18,21, X and Y using direct labelled chromosome specific alpha satellite or contig DNA using a 2 hour hybridization protocol. Second, three-probe, three-color FISH was performed for chromosomes 1, 16, and 22 using direct labelled alpha satellite DNA. Images were captured using a cooled CCD camera controlled by a MacIntosh computer using Image and FISH analysis software. **Results:**Twenty-six of 59 (44%) embryos were diploid. Four of 59 (6.8%) embryos were mosaic. Two trisomy 18's, one trisomy 13 or 21, and one XXY mosaic were observed. Three of 59 (5.1%) embryos were classified as chaotic. Tweny-four of 59 (40.7%) embryos, diagnosed by the analysis of just one blastomere, were either aneuploid or mosaic. Five trisomy 13 or 21's, three trisomy 18's, three Turner syndrome, one Klinefelter syndrome, two monosomy 13 or 21's, five trisomy 16's, one trisomy 1, and four trisomy 22's were observed. One embryo (1.7%) was triploid and one mis-diagnosis for a gonosome occurred (1.7%). **Conclusions:**Of the 59 embryos evaluated for preimplantation diagnosis, 54% were aneuploid, mosaic, chaotic or triploid. FISH analysis for chromosomes 1, 16, and 22 identified an additional 12.5% aneuploid embryos not detected when analyzed for chromosomes 13,18,21,X and Y.

Program Nr: 977 from the 1999 ASHG Annual Meeting

The C. elegans homologues of Ataxin-2 and Ataxin-2-Binding-Protein are essential genes in early development.
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Ataxin-2 , the gene product of the autosomal dominant Spinocerebellar Ataxia Type 2 (SCA2), is a protein of unknown function. Ataxin-2 interacts with a A2BP1, a member of a novel family of putative RNA-binding proteins. Since Ataxin-2 as well as A2BP1 are evolutionarily conserved we investigated the expression pattern and function in C. elegans. The human SCA2 gene has a 19.3 % amino acid identity to its homologue in C.elegans (CeAtaxin-2). Likewise, Ataxin-2-Binding Protein 1 (A2BP1) is 29.8 % identical to the homologous C.elegans protein (CeA2BP1). We studied the expression pattern of CeAtaxin-2 using the CeAtaxin-2 promotor coupled with a GFP expression vector. CeAtaxin-2 was widely expressed in the adult worm with strong expression in muscle. It was also heavily expressed in worm eggs. In order to elucidate the function of CeAtaxin-2 and CeA2BP1, we conducted RNA interference (RNAi) studies. RNAi leads to an effective knockout of the targeted gene, presumably by specific degradation of native mRNA. The interfering double-stranded RNA was introduced into larval L4 stage worms of the N2 strain by microinjection or soaking. Microinjection was carried out as a two-gonad injection. Soaking involved leaving the worms in in dsRNA-solution for 24 hours. In line with previous RNAi studies, microinjection was more effective than soaking. DsRNA representing the full length CeAtaxin-2 gene resulted in an early lethal phenotype in the offspring of all 40 microinjected worms. RNAi for the full length CeA2BP1 showed a similar arrest in development when 40 worms were injected. However, no effect was observed with either construct on the parental animals. We conclude that both CeAtaxin-2 and CeA2BP1 are essential cellular genes in the early development of C.elegans. Both are highly conserved in mammals. The fact that both genes give an early lethal phenotype when targeted by RNAi underlines previous findings regarding the interaction of Ataxin-2 and A2BP1.

Program Nr: 978 from the 1999 ASHG Annual Meeting

RHD/CE genotyping by polymerase chain reaction using sequence-specific primer in RHD negative Korean donors. *Y.T. Kim[1,4], S.B. Kim[4], Y.H. Lee[1], S.H. Park[3], Y.S. Whang[5], D.H. Seo[5], Y.J. Cho[2].* 1) Dept. of Obstetrics and Gynecology, College of Medicine, Korea Univ., Anam Hospital, Seoul, Korea; 2) Dept. of Clinical Pathology, College of Medicine, Korea Univ., Anam Hospital, Seoul, Korea; 3) Department of Pediatrics, College of Medicine, Korea Univ., Anam Hospital, Seoul, Korea; 4) Graduate School of Biotechnology, Korea Univ., Seoul, Korea; 5) Research Institute for Blood Transfusion, The Republic of Korea National Red Cross, Seoul, Korea.

Antigens of Rh blood group system are considered to be encoded on chromosome 1p34-36 by 2 genes, RHD and RHCE. In Caucasians, almost all RhD negative people have deletion in RHD gene, but in Orientals, such as the Japanese, up to 27.7% of RhD negative donors retained the RHD gene.This study was to confirm the incidence of RHD gene deletion in RhD negative Korean donors. After phenotyping 250 RhD positive and 116 negative Korean donors using polyclonal anti-C,c,E,e,D, we performed RCR-SSP to determine the deletion in exon 3,4,5,6,7,9,10, intron4, and intron4-exon5 boundary.

Phenotypes of 250 RhD positive consisted of 107 CCee(42.4%), 93 CcEe(37.2%), 26 ccEE(10.4%), 21 Ccee(8.4%), 3 ccEe(1.2%) and 1 ccee(0.4%). In 116 RhD negative donors, the frequency of phenotypes was 62 ccee(53.5%), 35 Ccee(30.2%), 9 ccEe(7.8%), 6 CcEe(5.2%), 3 CCEE(1.7%), and 3 CCee(1.7%). After genotyping, 22 samples (22%: 22/101) from RhD negative donors revealed intact RHD gene. Deletion was 76(75.3%) in intron4-exon5 boundary, 74(73.3%) in intron 4, 72(71.3%) in exon 4, 67(66.3%) in exon 7, 63(62.4%) in exon 10, and 9 (8.9%) in exon 5, but no deletion was found in exon 3,6,9.

We also compared the deletion frequency in various RHCE phenotypes of RhD negative donors. Over 90% in ccee showed deletion in exon 4,7,10, and inton 4 except for exon 5(9.4%). About 70% was deleted in ccEe; about 45% in Ccee; about 40% in CcEe. In conclusion, our result could provide useful data for prenatal diagnosis in RhD negative Korean pregnant women.

Program Nr: 979 from the 1999 ASHG Annual Meeting

Genetic Susceptibility to Preeclampsia: Roles of Factor V Leiden and Lipoprotein Lipase mutations. *Y.J. Kim[1], J. Andrews[1], P. Peraud[1], R.A. Williamson[1], J.C. Murray[1], D.C. Merrill[2].* 1) University of Iowa, Iowa City, IA; 2) Wake Forest University, Winston-Salem, NC.

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 the Factor V Arg506Gln (Factor V Leiden) and the lipoprotein lipase(LPL) Asp9Asn and -93G promoter mutations. DNA was extracted from whole blood or cheek swab of 320 preeclamptic patients and 260 controls (All Caucasians). Controls consisted of women who had undergone at least two term pregnancies unaffected by PRE. All samples were genotyped for all the polymorphisms using PCR of known allelic varients. Sequences were confirmed on an Applied Biosystems 373 DNA Sequencer. Results were analyzed with a X^2 contingency table. Seventeen of 310 women with PRE (5.5%) and 5 of 139 with severe PRE(3.6%) were heterozygous for the Leiden mutation compared with 12 of 254 controls (4.7%)(P=NS). Nine of 314 women with PRE (2.9%) and 5 of 141 with severe PRE (3.5%) were heterozygous for the lipoprotein lipase Asp9Asn mutation compared with 9 of 249 controls (3.6%). 15 of 306 women with PRE (4.9%) and 5 of 131 with severe PRE (3.7%)were heterozygous for the lipoprotein lipase -93G promoter mutation compared with 13 of 254 controls (5.1%). There were no significant differences between the groups for the lipoprotein lipase Asp9Asn mutation and -93G promoter mutation. No patients were homozygous for the Leiden mutation or the lipoprotein lipase mutations. There were also no significant differences identified when the genotype of the infant from the affected pregnancy was considered the case. Combining the rare variants for LPL Asp9Asn, LPL -93G promoter mutation, and Factor V Leiden (12.1% cases vs 11.3% controls) or stratifying the cases by severity did not affect significance. Our data suggest that carriers of the Factor V Leiden mutation, lipoprotein lipase Asp9Asn mutation, and -93G promoter mutation are not associated with an increased risk for preeclampsia. Additional analysis is being extended to family based controls, sib pairs, and new loci.

Program Nr: 980 from the 1999 ASHG Annual Meeting

Delineation of the paternal disomy 14 syndrome: Identification of a case by prenatal diagnosis. *J. Klein[1], L.G. Shaffer[2], C. McCaskill[2], L. Scheerer[1], C. Otto[1], D. Main[1], M. Thangaveul[3], J. Goldberg[1]*. 1) Prenatal Diagnosis, California Pacific Medical Ctr, San Francisco, CA; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) Genzyme Genetics, Orange, CA.

Uniparental disomy (UPD) may lead to a disease phenotype due to the effects of genomic imprinting or homozygosity of recessive alleles. We report a case of paternal isodisomy 14, the fifth reported to date. A G1P0 30 yo woman was referred for a positive maternal serum triple screen which predicted a 1/60 risk for Down syndrome. Ultrasound showed polyhydramnios, bilateral pyelectasis and hand contractures. An echocardiogram revealed an aortic arch anomaly. Cytogenetic analysis of cultured amniotic fluid revealed a 45,XX,der(14;14)(q10;q10)de novo karyotype. The pregnancy was terminated based on the ultrasound findings. DNA analysis of the fetus revealed homozygosity for 5 polymorphic markers on chromosome 14, with all alleles paternally inherited. There was no evidence of trisomy 14 mosaicism by cytogenetic or DNA analysis. Based on the molecular findings, the likely mechanism was a nullisomy 14 ovum fertilized by a normal haploid sperm. A postzygotic mitotic duplication, or monosomy rescue, resulted in formation of an isochromosome 14 and paternal UPD. The consistent prenatal features seen in our patient, as compared to others, appear to be polyhydramnios and hand contractures. Other features seen with this syndrome include skeletal anomalies and dysmorphic features. Our case is unique because of the heart anomalies and positive prenatal screen and thus should be considered in future cases. The severe phenotype in paternal disomy 14 is likely due to imprinted genes on chromosome 14 and the variability of some features may be due to homozygosity of mutant recessive alleles. Paternal disomy 14 should be considered in the presence of polyhydramnios and contractures, and in apparently balanced Robertsonian translocations involving chromosome 14. The prognosis of paternal UPD 14 appears to be uniformly poor. This case provides further evidence of a clinically relevant and consistent paternal UPD 14 syndrome.

Program Nr: 981 from the 1999 ASHG Annual Meeting

Determination of the absolute number of fetal cells in maternal blood using molecular cytogenetic techniques. *K. Krabchi, F. Gros-Louis, J. Yan, M. Bronsard, P. De Grandpre, J. Masse, J.-C. Forest, R. Drouin.* Pav Saint-Francois d'Assise, CHUQ, Dept Medical Biology, Laval University, Quebec, Quebec, CANADA.

The presence of fetal cells in maternal blood was first shown almost a century ago. Even though, many investigators have attempted to estimate the number of fetal cells present in maternal blood, there is still much controversy concerning the exact number of these cells. Therefore, the purpose of our project was to determine the absolute number of all the different types of fetal cells per unit volume of maternal blood. Peripheral blood samples were obtained from pregnant women between 18th to 22nd weeks of gestation. The selection of women known to be carrying male fetuses was necessary to recognize fetal cells (XY) among maternal cells (XX). Identification of fetal cells was carried out by fluorescent in situ hybridization (FISH) and by primed in situ labeling (PRINS) techniques. FISH using specific sex chromosome probes and PRINS using specific oligonucleotide primers and subsequent primer extension by Taq DNA polymerase were performed and fetal cells were identified and scored by fluorescent microscopy screening. Both techniques are highly specific and sensitive. However, the cheaper PRINS technique allows a more rapid detection compared to FISH. So far, six maternal blood samples have been analyzed without any enrichment procedures. The results showed that the range of fetal cells in maternal circulation fluctuates between 3 to 5 cells per ml of blood. Thus, we can conclude that it is possible to identify an extremely low number of fetal cells among millions of maternal cells. Moreover, these findings will allow the study of various factors that influence the number of fetal cells in maternal blood such as gestational age, status of mothers health (eg: diabetus, preeclampsia), smoking and drinking habits, ultrasound and chromosome constitution of the fetus. It is likely that the complex enrichment procedures actually under investigation for prenatal diagnosis of trisomy 21 cause the loss of fetal cells. Therefore, the estimation of absolute number of fetal cells constitutes critical information to obtain in order to assess the efficiency of an enrichment procedure.

Program Nr: 982 from the 1999 ASHG Annual Meeting

Use of Chorionicity to Refine the Risk of Chromosomal Abnormalities in Twin Pregnancy. *D.A. Krantz[1], T.W. Hallahan[1], J.W. Larsen, Jr.[2], P.D. Buchanan[3], R.J.M. Snijders[4], V. Klein[5], J.N. Macri[1].* 1) NTD Laboratories,Huntington Stat, NY; 2) The George Washington Univ., Washington, DC; 3) GeneCare Medical Genetics Center, Chapel Hill, NC; 4) Cedar Sinai Medical Center, Los Angeles CA; 5) Norh Shore Univ. Hospital, Manhasset, NY.

Information regarding the risk of chromosomal abnormality in one or both fetuses in a twin pregnancy assists patients in making decisions regarding invasive diagnostic procedures. Risks can be determined based on the percentage of twins that are dizygotic (Rodi et al 1990). However, zygosity can not easily be determined at this stage of pregnancy. Chorionicity is not synonymous with but can be used to give an indication of zygosity. A 10-14 week scan can determine dichorionicity (lambda-sign or twin peak) or monochorionicity (T-sign) by paying specific attention to the junction of the intertwin membrane and placenta. It is estimated that about 2/3 of twins are dizygotic and 1/3 are monozygotic. All dizygotic twins are dichorionic. Of monozygotic twins, about 2/3 are monochorionic and 1/3 are dichorionic. These figures may vary depending on other factors such as ethnicity or if twinning is spontaneous or induced. Bayes' rule can be used to determine the likelihood of zygosity based on chorionicity. Using the above percentages, all monochorionic pregnancies are monozygotic while 86% of dichorionic pregnancies are dizygotic. The table below gives an example of how chorionicity determined from U/S can refine the risk of Down syndrome for a 32 year old patient:

	Singleton	Twins	Monochorionic	Dichorionic
At least 1 Affected	1/659	1/395	1/659	1/355
Both Affected	-----	1/1991	1/659	1/4571

This approach could be developed for Down syndrome screening based on biochemistry and NT as well as maternal age.

Program Nr: 983 from the 1999 ASHG Annual Meeting

Preimplantation diagnosis for patients with homozygous beta-thalassemias. *A. Kuliev[1], S. Rechitsky[1], O. Verlinsky[1], V. Ivakhnenko[1], M. Angastiniotis[2], G. Kalakoutis[2], A. Kalnakou[2], N. Ginsberg[1], C. Strom[1], Y. Verlinsky[1]*. 1) Reproductive Genetics Inst, Chicago, IL; 2) Cyprus Thalassemia Center, Cyprus.

Preimplantation genetic diagnosis (PGD) is an important option for couples with one of the partners homozygous for beta-thalassemia, because of their 50% risk of having affected child. We performed PGD for two such couples, in one of whom female partner was homozygous for IVS1-110, and in the other - double heterozygous, IVS1-6/IVS1-110. In the latter couple, who attempted two subsequent PGD cycles, husband was a carrier of IVSII-745 mutation while in the former, paternal mutation was similar to the maternal one. As half of the resulting embryos from such couples were expected to have a normal paternal allele of the beta-globin gene, our strategy was to pre-select heterozygous embryos, using blastomere biopsy at the cleavage stage, coupled with nested PCR analysis and restriction digestion. To avoid a possible misdiagnosis due to contamination with extraneous DNA, or allele drop out, two highly polymorphic linked markers, STR at the 5' end of the globin gene and HUMTH01, which is a syntenic STR, were amplified simultaneously with beta-globin gene. This also required a single sperm typing to reveal the haplotypes of the affected and unaffected paternal chromosomes, prior to blastomere testing. Of a total of 10 embryos studied from both of these couples, 6 contained a normal allele, and transferred back to patients (3 embryos in one couple and 3 in the other), while the rest were used for a followed up study, which confirmed the affected status of these embryos. Overall, we performed 37 PGD cycles for different beta-globin gene mutations, resulting in pre-selection and transfer of unaffected embryos in each of these cycles. This resulted in establishment of 11 unaffected pregnancies, and birth of 6 healthy children. The follow up study of a total of 116 affected embryos rejected from transfer showed 98% accuracy of PGD.

Program Nr: 984 from the 1999 ASHG Annual Meeting

Prenatal detection of Charcot-Marie-Tooth disease type 1A duplication resulting from a rare recombination event. *V. Labelle[1], R.B. Bernard[1], S. Tardieu[2], J.P.. Azulay[3], P. Malzac[1], E. Leguern[2], N. Philip[1,4], N. Lévy[1,4]*. 1) Département de Génétique Médicale, Hôpital d'enfants de la Timone, Marseille; 2) Clinique des maladies neuromusculaires, Hôpital Timone, Marseille; 3) Inserm U289, Hôpital Pitié-Salpétrière, Paris; 4) Inserm U491 "Génétique Médicale et Développement, Marseille. FRANCE.

CMT1A is caused in most cases by a 1.5 Mb duplication arising after unequal crossing-over between repeated sequences (*CMT1A-REPs*), flanking the 1.5 Mb unit. Reiter et al. and Lopes et al. (1996), described a recombination "hotspot" within a 3.2 Kb junction fragment between *Eco*RI (distal *CMT1A-REP*) and *Sac*I (proximal *CMT1A-REP*), then reduced to 1.7 Kb between EcoRI and NsiI (Reiter et al.,1996) and recently to a 731 bp "hotspot" region within the *Eco*RI/*Nsi*I fragment (Lopes et al., 1998). We report a 28-year-old woman requesting prenatal diagnosis because of CMT1A familial history in her husband's family. The duplication was initially evidenced in the father's family by Southern blot analysis showing a 3.2 Kb *Eco*RI/*Sac*I junction fragment segregating with the duplication. Here we used a *CMT1A-REPs* based PCR strategy to characterize the foetus's status, since this approach detects the entire set of duplications occuring after recombination within the "hotspot". Surprisingly, the expected 1.7 kb (*Eco*RI/*Nsi*I) junction fragment was absent from the father's and foetus's DNAs. After *Eco*RI/*Sac*I digestion of the PCR product, a 3.2 Kb fragment was observed in their DNAs but not in mother and controls, indicating the duplication originated from a 1.5 Kb rare recombination zone. Although the karyotype was normal in the foetus, parents decided to interrupt the pregnancy because of a possible severe phenotype. Indeed, a large clinical heterogeneity was observed in this family. We report the interest of the *CMT1A-REPs* based PCR assay for use on trophoblastic tissues with obtention of results within 6 days. Opportunity and feasibility of preimplantation diagnosis will be compared to the ability of early prenatal diagnosis, to limit psychological distress due to pregnancy termination, particularly in diseases with unpredictible phenotype's severity.

Program Nr: 985 from the 1999 ASHG Annual Meeting

Influence of VPA and CBZ Monotherapy on Homocysteine Metabolism and Copper Levels in Premenopausal Epileptic Women. *D.M. Lambert[1,5], G.E. Graham[1,5], R. Rozen[2,5], B. Gilfix[3,5], H. Seni[4,5], E. Andermann[4,5], E.P. Treacy[1,5]*. 1) F Clarke Fraser Clinical Genetics Unit, CR Scriver Biochemical Genetics Unit and DeBelle Laboratory, Montreal Children's Hospital; 2) Montreal Children's Hospital Research Institute; 3) Royal Victoria Hospital; 4) Montreal Neurological Institute; 5) McGill University, Montreal, Canada.

Remethylation of homocysteine (hcys) to methionine (met) is essential for the production of s-adenosyl methionine and for other methylation reactions likely to be critical in embryogenesis. We postulated that anticonvulsants might exert their known teratogenic action by an effect on the hcys-met axis, influenced by folate/B12 status and MTHFR genotype. To establish whether the met/hcys (M/H) ratio was altered by VPA or CBZ monotherapy, we measured levels of met, hcys, RBC folate, B12, copper, zinc and anticonvulsants in 24 premenopausal epileptic women on long-term VPA or CBZ monotherapy and compared them to a control group of 11 healthy premenopausal women. All measurements were performed at midday following a minimum 3 hour fast to control for diurnal variation, and all participants were genotyped for the MTHFR thermolabile polymorphism C677T. We did not detect any homozygotes for the C677T polymorphism, and the prevalence of heterozygosity was similar between cases and controls (p=0.96). We did not detect a significant difference in M/H ratio between cases and controls, and methionine was significantly higher in cases (20.4 vs.16.2; F^1_{32}; p=0.005). The M/H ratio was independent of the dose of anticonvulsant (F^2_{20}; p=0.86). However, among CBZ cases, heterozygotes for the MTHFR polymorphism had significantly lower M/H ratios (p=0.03). No such relationship was observed in the VPA or control groups. Multivariate analysis indicated that M/H ratio was best predicted by the presence and type of medication, and the presence or absence of MTHFR polymorphism (F^2_{30}; p=0.04; R^2=0.19). Maternal copper deficiency has been associated with birth defects; thus of particular interest, serum copper was found to be significantly lower in both the VPA and CBZ groups compared to controls (F^1_{32}; p=0.0004) and was inversely correlated with M/H ratio (p=0.05).

Program Nr: 986 from the 1999 ASHG Annual Meeting

Hyperechogenic fetal bowel: an ultrasonographic marker associated with low risk of cystic fibrosis. *S.F. Langlois[1], F. Tessier[2], F. Sizmur[2], L.C. Kwong[1], C.R. Siemens[1], R.D. Wilson[1,2]*. 1) Dept Medical Genetics, Univ British Columbia, Vancouver, BC, Canada; 2) Dept Obstetrics and Gynecology, Univ British Columbia, Vancouver.

Ultrasonographic detection of fetal hyperechogenic bowel is associated with an increased risk of cystic fibrosis (CF), aneuploidy and gastrointestinal malformation. The incidence of CF in this patient population varies from 0-20% in published studies. To determine the risk of CF in our population, we reviewed all cases referred to the Molecular Diagnostic Laboratory for CF testing because of an ultrasound finding suggestive of an increased risk of CF in the fetus.

129 pregnant patients were studied. In 116 cases, the partner was also tested. 208 individuals were of European descent. Of the 129 cases, 94 patients were referred for hyperechogenic bowel (echogenecity less than bone in 11, equal or greater than bone in 68 and no grade indicated in 15 cases). In 17 cases, echogenic calcifications were seen suggestive of meconium peritonitis. In an additional 10 cases, associated dilated bowel loop(s)were seen. Finally 8 cases were tested because of an echogenic mass or echogenic cyst.

Mutation analysis for 12 common CFTR mutation detected 5 carrier parents. In all 5 cases, the partner was negative. Four cases were referred for echogenic bowel and one case, for echogenic bowel with dilated bowel loop. In one case, testing of amniocytes showed that the fetus had not inherited the parental mutation; in three cases the fetus did inherit the parental mutation but investigations in the newborn period excluded the diagnosis of CF. The last case (echogenic bowel with dilated bowel loop) did not have testing of amniocytes and was lost to follow up.

In summary, no case of cystic fibrosis was diagnosed amongst the 94 cases referred for isolated hyperechogenic bowel. Amongst this group, three fetuses were found to be CF carriers, an incidence consistent with the population frequency of CF. A review of neonatal outcomes will be discussed.

Program Nr: 987 from the 1999 ASHG Annual Meeting

Abnormal multiple marker screen and ultrasound findings of fetal edema secondary to Congenital Erythropoietic Porphyria. *N. Lazebnik[1,2], C. Tsai[1].* 1) Dept Genetics, Henry Ford Hosp, Detroit, MI; 2) Dept OB/GYN, Henry Ford Hosp, Detroit, MI.

A Caucasian couple of western European decent, a 22-year-old G1P0 female and her 27-year-old husband were initially referred for genetic consolation due to an abnormal multiple marker screen at 16 weeks (MSAFP 2.87 MoM, hCG 1.5 MoM, and uE3 0.39). A level II ultrasound study was remarkable for a 9.7 mm nuchal fold, skin edema, mild ascites, and pericardial effusion. Amniocentesis documented a normal female karyotype, 46, XX, and normal AFAFP. TORCH studies were negative. Follow up ultrasound study at 31 weeks documented adequate fetal growth, mild ascites, pericardial effusion, skin edema and a mildly larger than expected heart. The liver dimensions were >90%. The placenta was thicker than normal (5.9 cm). The patient was induced to labor at 34 weeks gestation, due to decreased amniotic fluid volume and fetal heart rate decelerations. The Apgar scores were 6 and 7 at 1 and 5 minutes respectively. Birth weight was 2089 g (31st percentile). The newborn physical examination was remarkable for generalized hydrops, cloudy corneas, glaucoma, bilateral semian crease, and small hypoplastic nails. In addition severe hemolytic anemia, pancytopenia, massive hepatosplenomegaly, and skin blistering in areas exposed to light were noted. Due to severe respiratory distress the newborn was started on ECHMO. Comprehensive hematology work up documented Congenital Erythropoietic Porphyria secondary to Uroporphyinogen III Synthase deficiency. Molecular study documented homozygosity for the C73R mutation, a severe transfusion-dependent phenotype. The results were confirmed by showing that each parent has one copy of the C73R mutation. Recently the patient underwent bone marrow transplantation.

Program Nr: 988 from the 1999 ASHG Annual Meeting

The N terminal sequence of PMP70 is sufficient for targeting to peroxisomal membrane in CHO cells but not in S. cerevisiae. *S. Almashanu*[1,2], *D. Valle*[1,2]. 1) Howard Hughes Medical Institute; 2) Johns Hopkins University School of Medicine, Baltimore, MD.

The 70 kD peroxisomal membrane protein, PMP70, is one of four (PMP70, P70R, ALDP, ALDR) half ATP binding cassette (ABC) transporters targeted to human peroxisomal membranes by a poorly understood pathway. All of these have six transmembrane domains (TMD) in their N-terminal half with a hydrophobic C-terminal half containing an ATP binding domain. To identify targeting motifs, we generated a series of N-terminal and C-terminal deletions of PMP70 tagged with a C-terminal green fluorescent protein (GFP) and expressed them in Chinese hamster ovary (CHO) cells. We localized a peroxisomal membrane targeting signal to the 80 N-terminal amino acids, preceding the first TMD. We made a chimeric protein with the N-terminal 183 residues of PMP70 followed by the C-terminal 428 residues of its E. coli homolog (YDDA) tagged with GFP. In contrast to YDDA-GFP alone which showed a nonperoxisomal pattern, the chimeric PMP70/YDDA-GFP localizes to peroxisomes. Thus the N-terminus of PMP70 is both necessary and sufficient for peroxisomal targeting. To study targeting in a more malleable system, we expressed each of the four human peroxisomal membrane half ABC transporters in S. cerevisiae with high levels of the corresponding mRNA but no detectable protein. Similarly, when we expressed the truncated PMP70 GFP fusions that localize to peroxisomes in CHO cells, we observed a diffuse cytosolic GFP pattern in yeast. These results suggest some difference in the targeting pathway of peroxisomal half ABC transporters between humans and yeast. Recently, several human peroxins (PEX19, PEX16, PEX3) and yeast peroxins (PEX19 and PEX3) have been suggested to play a crucial role in the targeting of PMPs. One difference is an apparent absence of a PEX16 homolog in S. cerevisiae. We are currently attempting to reconstitute the human targeting pathway in S. cerevisiae by introducing all human genes potentially involved in this pathway (PEX19, PEX16, PEX3) and determining their effect on PMP70 targeting.

Program Nr: 989 from the 1999 ASHG Annual Meeting

The Identification of Twenty-One SNPs in Xq and Candidate Gene Analysis in Rett Syndrome. *R. Amir[1], E.J. Roth Dahle[3], H.Y. Zoghbi[1,2,3].* 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas; 2) Pediatrics, Baylor College of Medicine, Houston, Texas; 3) Howard Hughes Medical Institute, Baylor College of Medicine, Houston, Texas.

Rett syndrome (RTT) is a neurodevelopmental disorder primarily affecting females. Most cases are sporadic but, several reports about familial recurrence support X-linked dominant inheritance with male lethality. RTT families were used for mapping studies and resulted in the exclusion of large portions of the X chromosome except for a small region on Xp22.31 and the distal Xq from Xq25-Xqter. Recently, exclusion mapping studies using a new family defined Xq28 as the candidate region for the RTT gene. Accordingly, six candidate genes were selected for mutation analysis based on their established expression patterns and known functions in the CNS. These are: Glutamate receptor subunit 3 (*GLUR3*), GABA receptor subunit alpha 3 (*GABRA3*), GABA receptor subunit e1 (*GABRE1*), Vacuolar ATPase subunit 1 (*VATPS1*, *XAP3*), the human homologue of plexin 3-"*SEX*" (*XAP6*) and the Synaptobrevin-like protein (*SYBL1*). Major rearrangements involving these genes were excluded by Southern analysis. The genomic structures of *GLUR3* and *GABRA3* were established. Genomic DNA from 30-50 RTT patients were screened for mutations by PCR amplification of all coding exons as well as the 5' and 3' untranslated regions, followed by Conformation Sensitive Gel Electrophoresis (CSGE) analysis and direct sequencing. The "*SEX*" gene was analyzed by direct sequencing of RT-PCR products amplified from RNA derived from RTT patients. No disease-causing mutations were found but twenty one Single-Nucleotide Polymorphisms (SNPs) were detected in the six genes studied. The SNPs identified will be useful in future linkage analysis and whole-genome association studies for other diseases. The genomic characterization of *GLUR3* and *GABRA3* will allow mutational analysis of these genes as candidates for other X-linked neurological disorders mapping to Xq25-Xq26 and Xq28. As the candidate region harboring the RTT gene is now localized to the most distal band of Xq28, our future efforts will focus on the analysis of additional candidate genes in this region.

Program Nr: 990 from the 1999 ASHG Annual Meeting

Effect of a *CYP3A4* Promoter Variant on a Reporter Gene. *B. Amirimani[1,2], A.H. Walker[2], B.L. Weber[1,3], T.R. Rebbeck[2]*. 1) Department of Medicine, University of Pennsylvania, Philadelphia, PA; 2) Department of Clinical Epidemiology and Biostatistics, University of Pennsylvania, Philadelphia, PA; 3) Department of Genetics, University of Pennsylvania, Philadelphia, PA.

Of the P450 cytochromes, CYP3A4 is the major metabolizer of carcinogens, drugs, and steroid hormones in adult liver. We have previously identified a variant in the promoter region of *CYP3A4*, and reported that it may be associated with cancer susceptibility. To evaluate the functional significance of this variant, we generated two transfection vector contructs, each containing 1 kb of the *CYP3A4* promoter, an SV40 enhancer element, and a luciferase reporter gene. One construct contained the wild-type (*W*) *CYP3A4* promoter sequence, and the other contained the variant (*V*) *CYP3A4* promoter sequence. The expression of these promoter constructs was studied in HepG2 and MCF7 cell lines. In HepG2, luciferase expression was 1.6 fold higher in *V* compared with *W* promoter ($p<.0001$). In MCF7, luciferase expression was 2 fold higher in *V* compared with *W* promoter ($p<.0005$). These results suggest that *CYP3A4* transcriptional expression may be higher in the presence of a *V* promoter than a *W* promoter. Since CYP3A4 is an important metabolizer of numerous human carcinogens and steroid hormones, this genetic variant may explain interindividual variability to cancer susceptibility and drug metabolism.

Program Nr: 991 from the 1999 ASHG Annual Meeting

Analysis of variable TIMP1 expression from the inactive X chromosome. *C. Anderson, C. Brown.* Dept Medical Genetics, Univ British Columbia, Vancouver, Canada.

X inactivation silences most of the genes on one of the two Xs in females. We have shown that the human TIMP1 gene has polymorphic X inactivation - being expressed from the inactive X in some females but not others. As methylation is generally correlated with X inactivation, we examined methylation of 3 sites at the 5 end of TIMP1. Methylation was detected in females, presumably due to the presence of an inactive X but not in males who have only one active copy. However, methylation was still observed in females with TIMP1 expression from the inactive X, suggesting methylation is not sufficient to inactivate TIMP1. To analyze the factors involved in TIMP1 inactivation, we used somatic cell hybrids where TIMP1 expression from the inactive X has also been observed. Expression from the inactive X was limited to the TIMP1 gene as flanking genes remained silent. Two of three inactive X hybrids expressing TIMP1 showed methylation. This may have reflected a heterogeneous population of cells so single cell clones from all of the inactive X hybrids expressing TIMP1 were analyzed. In the hybrids unmethylated at the 5 end of TIMP1, all 13 clones were unmethylated and expressing TIMP1, indicating stable expression. The levels of TIMP1 expression were similar to the expression levels seen in active X hybrids. However, in the hybrids that had retained methylation at the 5 end of TIMP1, 8 of 25 clones expressed TIMP1 and remained methylated with a level of expression below that of active X hybrids. Therefore, it seems that methylation is not sufficient for silencing but still has a suppressive effect. To further examine the role of methylation, we demethylated inactive X hybrids that originally did not express TIMP1. Seven clones reactivated TIMP1 and surrounding genes were also expressed, in contrast to the gene-specific expression observed for spontaneous TIMP1 expression from the inactive X. Since methylation does not seem to be the only factor affecting TIMP1 expression from the inactive X, we are currently analyzing the contribution of expression levels to stability and examining the role of regional chromatin configuration in maintaining TIMP1 inactivation.

Program Nr: 992 from the 1999 ASHG Annual Meeting

Identification of *Barhl1*, a novel homeobox gene of the *Bar* family highly expressed in the primordia of the cerebellum. *S. Banfi, E. Menguzzato, V. Broccoli, A. Marchitiello, C. Gattuso, A. Ballabio, A. Bulfone.* Telethon Institute of Genetics and Medicine (TIGEM), San Raffaele Biomedical Science Park, Milan, Italy.

The *Drosophila* dual *Bar* genes (*BarH1* and *BarH2*) encode two homeodomain-containing proteins which play an important role in the development of the external sensory organs in the fly. In the course of a project aimed at the systematic identification of human and/or murine cDNAs similar to *Drosophila* mutant genes in dbEST, we identified *Dres115*, a murine EST showing a very high sequence homology to the *Drosophila Bar* genes. By cDNA library screening, we isolated and sequenced the full-length *Dres115* cDNA, subsequently renamed *Barh-like1 (Barhl1)*. We also identified and mapped the corresponding human *BARHL1* gene. The murine transcript is predicted to encode a 327-amino acid homeodomain protein which shows the highest sequence similarity with the BarH1 and BarH2 *Drosophila* proteins and the recently identified rat mBH1 protein. To study the expression pattern of the Barhl1 gene in detail, we decided to carry out RNA *in situ* hybridization experiments in mouse, on both whole embryos and tissue sections. These studies showed that *Barhl1* is expressed at remarkable levels and at very early stages in the developing central nervous system (CNS). The main domains of expression are represented by the pretectum in the diencephalon, the primordia of the cerebellum and the most dorsal portion of the alar plate in the rhombencephalon, and in the spinal cord. In postnatal stages, *Barhl1* is only detected in the cerebellar cortex, specifically in the external granular layer. This intriguing expression pattern suggests that *Barhl1* might play a role in the development of the vertebrate CNS in both normal and pathological conditions.

Program Nr: 993 from the 1999 ASHG Annual Meeting

CASTing for genes regulated by PAX3 during embryonic development and cancer epigenesis. *T.D. Barber[1,2], T.B. Friedman[1]*. 1) NIDCD, NIH, Rockville, MD; 2) Genetics Program, MSU, East Lansing, MI.

PAX3 is a transcription factor important for neural, muscle, facial and auditory development in vertebrates. Mutations in PAX3 cause Waardenburg syndrome (WS) types 1 and 3 and Craniofacial-deafness-hand syndrome in humans and the Splotch phenotype in mice. A translocation t(2;13)(q35;q14) gives rise to a chimeric fusion protein, PAX3/FKHR, and results in alveolar rhabdomyosarcoma (ARMS). In order to identify genes regulated by PAX3 during embryogenesis and tumor formation, we have used a Cyclic Amplification and Selection of Targets (CASTing) strategy to isolate genes whose cis-regulatory elements are bound by PAX3. A library of mouse genomic fragments that bind Pax3 protein in vitro was generated, and additional libraries of human DNA fragments which interact with PAX3 and PAX3/FKHR are being constructed. Over 1000 clones from the mouse library have been sequenced, and approximately 100 genes have been identified thus far. We are beginning to screen these genes for Pax3-dependent expression patterns in Splotch mice and other model systems using northern, in situ and microarray hybridization analyses. Early results suggest that many of these putative Pax3 target genes have expression patterns and predicted functions consistent with regulation by Pax3. Two promising candidates include Itm2A and even-skipped 2. We have determined that Itm2A is abundantly expressed in developing murine skeletal muscle and is down-regulated in Splotch mutant embryos. Previous studies have shown that a Drosophila homolog of even-skipped 2 is regulated by paired (prd), a homolog of Pax3, suggesting that the regulation of even-skipped 2 by Pax3 has been conserved throughout evolution. Identification and characterization of genes regulated by PAX3 will provide a better understanding of the role of PAX3 in neural, muscle, auditory and craniofacial development, and how mutations in PAX3 perturb these processes in individuals with WS and ARMS.*Splotch, in vitro, in situ, Itm2A, even-skipped 2*.

Program Nr: 994 from the 1999 ASHG Annual Meeting

Heavy chain dynein DNAH9: cDNA sequence, genomic structure and exclusion as the gene responsible for one form of Primary Ciliary Dyskinesia. *L. Bartoloni[1], A. Maiti[1], J.L. Blouin[1], C. Rossier[1], M. Meeks[2], C. Gehrig[1], A.J. Sainsbury[1], C.D. DeLozier-Blanchet[1], M. Gardiner[2], H.S. Scott[1], S.E. Antonarakis[1].* 1) Division of Medical Genetics, University of Geneva Medical School, Switzerland; 2) Department of Pediatrics, University College London, UK.

Heavy chain dyneins are large proteins with ATPase activity involved in microtubule-mediated transport of organelles (cytoplasmic dyneins), and in motility of cilia and flagella (axonemal dyneins). We report the first complete cDNA sequence and genomic structure of a human axonemal dynein heavy chain gene, DNAH9, which maps to 17p12. The gene is divided into 69 exons over 400 Kb, with 14 Kb of coding sequence for a protein of 4488 predicted amino acids. The cDNA sequence of DNAH9 was determined using a combination of methods including: extension of previously determined partial sequences by 5'RACE, RT-PCR, and cDNA library screening. The predicted protein sequence is homologous to sea urchin axonemal heavy chain dyneins (67% identity). RT-PCR in nasal epithelium and testis revealed several alternatively-spliced transcripts. The genomic structure was determined using the publicly available sequences of 2 overlapping BACs produced by the Whitehead/MIT Genome Sequencing Center. These data provide tools for examining DNAH9 as a candidate gene for Primary Ciliary Dyskinesia (PCD), a recessive disorder with respiratory infections, bronchiectasis, male sterility and (dys)immotility of cilia; situs inversus is present in 50% of cases (Kartagener Syndrome). Ultrastructural defects of cilia are often found and they are related to the dynein complex in 50% of cases. Moreover a missense mutation in a heavy chain dynein gene was found in the iv/iv mouse, which has situs inversus. A highly informative dinucleotide polymorphism was found in intron 26. We used this polymorphism in 31 families with at least 2 siblings with PCD, and detected 2 families with co-segregation of both DNAH9 alleles in affecteds. Mutation search by direct sequence was performed in these 2 "candidate" families. We detected polymorphic variants but not a pathogenic mutation, thereby excluding DNAH9 as responsible for one form of PCD in our family sample.

Program Nr: 995 from the 1999 ASHG Annual Meeting

Characterization of the human *Talin* gene. *T. Ben-Yosef, C.A. Francomano.* Medical Genetics Branch, NHGRI/NIH, Bethesda, MD.

Talin is a high molecular weight cytoskeletal protein, localized at cell- extracellular matrix associations known as focal contacts. In these regions talin is thought to link integrin receptors to the actin cytoskeleton. Talin plays a key role in the assembly of actin filaments, and it is also essential for spreading and migration of various cell types.

Talin proteins are found in a wide variety of organisms, from slime molds to humans. The human gene was previously mapped to chromosome 9p, but little was known of its sequence and genomic structure. To further characterize human *Talin* we have isolated a single BAC clone, harboring the entire *Talin* gene. The gene extends over more than 23 Kb, and consists of 57 exons. Exon boundaries completely conform to the classical 5-donor and 3-acceptor consensus rules. We have localized *Talin* to human chromosome band 9p13 by both fluorescence in situ hybridization and Radiation Hybrid mapping. Northern blot analysis detected *Talin* expression in various human tissues, including peripheral blood leukocytes, lung, placenta, liver, kidney, spleen, thymus, colon, skeletal muscle and heart.

Based on its chromosomal location, expression pattern and protein function we considered *Talin* as a candidate gene for Cartilage-Hair Hypoplasia (CHH). CHH is an autosomal recessive metaphyseal chondrodysplasia characterized by short stature, hypoplastic hair growth and T-cell mediated immunodeficiency, which was previously mapped to 9p13. We sequenced the entire *Talin* coding sequence in several CHH patients, but no functional mutations were detected.

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Program Nr: 996 from the 1999 ASHG Annual Meeting

Genomic Structure of Human Oligophrenin-1 and its implication in the axon and dendrite outgrowth. *P. BILLUART, J. CHELLY, A. CARRIE, M.C. VINET, N. McDONELL, F. FAUCHEREAU, P. COUVERT, C. BELDJORD, T. BIENVENU.* INSERM U129, ICGM, CHU Cochin, Paris, FRANCE.

We have recently shown that mutations in oligophrenin-1 (OPHN1) are responsible for nonspecific X-linked mental retardation. The structure of the gene encoding the OPHN1 protein was determined by isolation of genomic DNA clones from a human cosmid library. Genomic fragments containing exons were sequenced, and the sequences of the exons and flanking introns were defined. Knowledge of the genomic structure of OPHN1 gene, which spans at least 500 kb and consists of 25 exons, will facilitate the search for additional mutations in OPHN1. OPHN1 encodes a protein with a relative molecular mass of 91 KDa which contains a domain typical of rho-GTPase-activating proteins. Further investigations using polyclonal antibodies allowed us to define the cellular localisation in primary cultures of neuronal and glial cells. Interestingly, a cell type-dependant localisation of OPHN1 and a potential interaction with cytoskeletal proteins were pointed out. Also, a significant immunolabeling was observed in growth cones of cellular neurites. These data are coherent with our hypothesis that OPHN1 could be involved in the outgrowth of axons and dendrites in vivo. Therefore, a defect in neurite outgrowth and in the establishment of connections between neurones could be the cellular basis underlying cognitive impairment.

Program Nr: 997 from the 1999 ASHG Annual Meeting

Isolation, expression and mapping of mCul3, the mouse ortholog of human CUL3 a member of the cullin/Cdc53 family. *H. Boettger-Tong[1], A.I. Agulnik[1], N. Roeckel[2], C.E. Bishop[1], N. Lévy[1,2]*. 1) Department of Obstetrics/Gynecology, Department of Human and Molecular Genetics, Baylor College of Medicine, 77030 Houston, TX 2; 2) Inserm U491 "Génétique Médicale et Développement, Faculté de Médecine, 27, Bd. Jean Moulin, 13385 Marseille Cedex, France.

While constructing a genetic map from mouse proximal chromosome 1, we identified a novel mouse gene homologous to Human Cullin 3 (hCUL3) a member of the cullin/Cdc53 family. We named this gene mCul-3 for Mus musculus Cullin 3. The full length cDNA was cloned; it encodes a 768 amino-acid polypeptide and shows 95% nucleotide and 99% amino acid identity to hCUL3. Here we show that mCul3 is transcribed in a wide range of tissues by RT-PCR, with a particularly high expression in testis as evaluated by Northern blot analysis. Sequencing analysis and protein prediction indicated that the mouse Cul3 homolog contained several motifs conserved between C.elegans, mouse and human. One of them, located at the N terminal region of the protein, is 32 amino acids long including peptides 40 to 71 and is identical between the 3 species. Several differences exist between the mouse and human orthologs : (i) In human, hCUL3 has been found to be ubiquitously transcribed with a predominant expression in skeletal muscle and heart. In the mouse, Cul3 is predominantly expressed in the testis; expression in other tissues is detectable by RT-PCR, but not by Northern blot analysis. (ii) hCul3 shows two different transcripts, respectively 2.8 Kb and 4.3 Kb; only a 2.8 Kb transcript was found in mouse. Cullins/Cdc53 are a recently identified family of genes/proteins whose six members have been characterized in human (Kipreos et al., 1996). Several studies suggested a role for the Cullin proteins in cell cycle regulation. Based on structural homology, it is likely that Cul3 is also involved in cell cycle proliferation. Based on the predominant expression pattern of the mouse gene in testis, and its nearly absent expression in ovary, we speculate that mCul3 may have a function in spermatogenesis.

Program Nr: 998 from the 1999 ASHG Annual Meeting

Identification of AHCP, a human gene expressed during brain development and candidate for genetic predisposition to schizophrenia. *T. Bourgeron[1], S. Jamain[1], M. Clergues[1], H. Quach[1], R.H. Segman[2], K. Kanias[2], M. Fellous[1], B. Lerer[2]*. 1) Lab Human Genetics, Institut Pasteur, Paris, France; 2) Biological Psychiatry Laboratory, Dept of Psychiatry Hadassah - Hebrew University Medical Center Ein Karem Jerusalem 91120, Israel.

We have isolated a human gene AHCP (Autosomal Highly Conserved Protein) localised on the human chromosome 6p. Analysis of the protein sequence indicates that AHCP is a putative membrane protein with no known consensus motifs. The genomic structure of the gene is conserved in mice and C.elegans. but D. melanogaster seems to have lost the introns. In human and mice, the gene is expressed ubiquitously in the adult with a smaller transcript specific to the testis. In mice, during development, the expression seems to be restricted to the forebrain. AHCP transcripts are found at the boundaries of the telencephalon and the diencephalon closed to the zona limitans intrathalamica (ZLI). In human, there are several retro-transcribed pseudo-genes including one copy on the Y chromosome. The localisation of the transcribed gene is between D6S274 and D6S285 on human chromosome 6p23. This region was found by different studies to contain a locus for predisposition to schizophrenia. In order to test the gene as a candidate, we have screened 73 schizophrenic patients for base substitution in the coding region. Three polymorphisms were found and the transmission disequilibrium test is under way.

Program Nr: 999 from the 1999 ASHG Annual Meeting

Role of human mitochondrial translocators in the import of nuclear encoded proteins. *A.M. Brown, W.T. Garvey, G. Argyropoulos.* Medicine, Medical Univ South Carolina, Charleston, SC.

Transfer of cytosolic preproteins into the mitochondria requires components of the Translocase machinery of the Outer mitochondrial Membrane (TOM) and immediate transient interaction with the Translocase machinery of the Inner mitochondrial Membrane (TIM). Initially, preproteins bind to receptors of the TOM complex and they are inserted into the intermembrane space via further binding to receptors of the General Import Pore. Final insertion of preproteins into the matrix is mediated by the various translocators of the inner membrane and the matrix heat shock protein 70. Over 20 protein members belonging to the TIM and TOM complexes have been identified in yeast but limited research has been done in mammalian systems and only a handful of TIM/TOM cDNAs have been cloned in humans (hTOM34, hTOM20, hTIM17, hTIM23, hTIM44, hTOM40, and hTIM8). The binding affinities and the requirement of hTIMs and hTOMs for the importation of human nuclear encoded mitochondrial proteins (NEMPs) (hUCP2, hUCP3, hBCL-XL, hBAK1, and hVDAC1) is being investigated and reported here. In vitro binding assays show that hTOM20 has binding affinities for hUCP2 and hUCP3 and hVDAC1. ELISA assays also indicate that a native mutant isoform of hUCP3 (R70W) has a significantly stronger binding affinity for hTOM20 than the wild type protein does. In addition, a novel translocator of the outer mitochondrial membrane has been identified and characterized. In organello experiments involving isolated mitochondria, in vitro purified hTIMs and hTOMs, and fluorescent-labeled NEMPs are also presented. The significance of mitochondrial translocators in metabolic diseases (obesity, diabetes) and their possible involvement in apoptotic events due to their interactions with NEMPs, are also discussed.*In organelloin vitro.*

Program Nr: 1000 from the 1999 ASHG Annual Meeting

Overexpression of a FANCD-EGFP fusion protein leads to accumulation in the nucleus and rapid cell death.*FANCD*. *D.A. Bruun, J.A. Hejna, C.D. Timmers, M. Grompe, R.E. Moses.* Department of Molecular & Medical Genetics, Oregon Health Sciences University, Portland, OR.

The Fanconi anemia complementation group D (FANCD) gene has recently been cloned. The full-length cDNA contains a 4413 bp open reading frame. Like the other cloned Fanconi anemia genes, the sequence reveals few identifiable features; however, the N-terminal region of the translated open reading frame is rich in Arg and Lys, suggesting a nuclear localization signal (NLS). Despite the absence of a consensus NLS motif, we decided to test whether this region could translocate an expressed green fluorescent protein fusion construct to the nucleus. A 2 kb fragment from the 5' end of the cDNA was ligated in-frame to pEGFP-N1 (ClonTech). Electroporation of the construct into GM639 fibroblasts resulted in a green fluorescent signal in only a small percentage (5%) of cells, compared to cells electroporated with pEGFP-N1. The cells expressing the fusion protein showed foci of green fluorescence in the nucleus. In addition, most of these cells appeared to be undergoing cell death, even within 16 hours after electroporation. In order to uncouple the cell-killing phenomenon from nuclear localization, a 200 bp fragment carrying the N-terminal 50 codons was ligated in-frame to pEGFP-N1. Electroporation of this construct into GM639 cells resulted in nuclear-localization of the fusion protein without cell death. The FANCD protein thus appears to carry a novel NLS and may function in an apoptotic pathway.*FANCD.*

Program Nr: 1001 from the 1999 ASHG Annual Meeting

From fish to human, structural and functional evolution of the p53 protein. *J. Cachot[1, 2, 3], F. Leboulenger[1, 3], T. Frebourg[2, 3]*. 1) Ecotoxicology Laboratory, University of Le Havre, Le Havre, FRANCE; 2) INSERM EPI 9906, Faculté de Médecine et de Pharmacie, Rouen, FRANCE; 3) IFRMP, Mont-St-Aignan, FRANCE.

Since its cloning in 1983, the p53 gene has been identified in about 35 vertebrates from fish to human. In mammals, this gene encodes a 53 kDa transcription factor implicated in cell cycle control, apoptosis and cellular responses to DNA damages and its inactivation plays a key role in tumorigenesis. The flounder (Teleostei, Pleuronectidae), a sentinel species for ecotoxicity, is one of the most phylogenetically distant species from human in which p53 has been cloned. The two p53 proteins display relatively low percentage of amino-acid identities within the transactivation (32%) and oligomerization (30%) domains, whereas the core DNA binding domain is much more conserved (57%). The functional assay developed in yeast to screen for p53 mutations in human tumors was used to test the biological activity of the flounder p53 protein. Preliminary results demonstrate that flounder p53 acts as a transcription factor which activates genes implicated in cell cycle control (p21) and apoptosis (Bax and PIG3). This transcriptional activity was highly dependent of temperature and flounder p53 optimal temperature was different from human p53, 20°C versus 30°C in human. These results are in complete agreement with biological data and reflect structural and functional adaptations of p53 proteins to physiological and environmental conditions of each species.

Program Nr: 1002 from the 1999 ASHG Annual Meeting

Evolution of the glucocerebrosidase pseudogene. *M. Callahan, R. Samimi, N. Tayebi, E. Sidransky.* DHHS/PHS, NIH/NIMH, Bethesda, MD.

Gaucher disease, the most common of the lysosomal storage disorders, is caused by mutations within the gene encoding for the enzyme glucocerebrosidase. Human glucocerebrosidase has a pseudogene located 16kb downstream on chromosome 1q21 that shares 96% homology with the functional gene. Many glucocerebrosidase mutations identified in patients with Gaucher disease are identical to pseudogene sequence, which suggests that the presence of the glucocerebrosidase pseudogene contributed to mutation introduction. Sequencing of the region surrounding the murine glucocerebrosidase gene demonstrated that this species does not have a nearby pseudogene. Two strategies were used to determine the presence or absence of a pseudogene in other species. After comparing the human and murine glucocerebrosidase and human pseudogene sequences, PCR primers were designed in conserved regions to enable the amplification of gene and pseudogene fragments whose size differs. Appropriate primers for two different glucocerebrosidase regions were identified, and the screening of DNA from nine different species indicated that the pseudogene was present in the human and rhesus monkey but absent from the mouse, rat, and chicken. Southern analysis using genomic DNA digested with SspI and Hind III confirmed the presence of the glucocerebrosidase gene and pseudogene in the human and monkey but not the other species. Further investigations using DNA sequencing are currently being performed.

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Program Nr: 1003 from the 1999 ASHG Annual Meeting

Molecular cloning and mapping on human chromosome 21q22.1 of a new human metalloproteinase: ADAMTS-1. *C. Casas, M. Pritchard, M.L. Arbones, X. Estvill.* Medical and Molecular Genetics, IRO, Barcelona, Spain.

An initial step in understanding the pathogenesis and pathophysiology of Down syndrome and the monogenic disorders and traits associated to chromosome 21 (HC21) is the cloning and characterization of genes located on HC21. As a part of the effort to identify new HC21 genes, we used an in silico approach and searched for ESTs at the chromosome 21-specific UniGene database and found a cluster of partial cDNAs with high similarity to the mouse gene Adamts-1. The murine gene was isolated by differential display from the RNA extracted from tumours induced in mouse by the injection of a colon 26 cachexigenic cell line. It encodes for a secreted metalloproteinase with thrombospondin type I (TSP-1) repeats that anchors to the extracellular matrix and seems to be involved in inflammatory processes. We present the isolation, characterization, expression pattern and mapping of the human orthologue of the murine Adamts-1 gene. Expression analysis of ADAMTS-1 by Northern showed a weak signal in muscle tissues, lung, placenta, peripheral nervous system and in discrete regions of the central nervous system, such as cerebellum and putamen. Analysis in depth of ADAMTS-1 predicted protein primary structure and comparison to other recently discovered matrix degrading proteases; the aggrecanase-1 and the procollagen-I-N-proteinase, confined them into a novel subfamily of metalloproteinases with TSP-1 repeats, the ADAMTS (A Metalloproteinase And ThromboSpondin). Since adhesion and proteolysis of the extracellular matrix are vital processes for morphogenesis, cell migration, tissue repair and cell death, an abnormal expression of ADAMTS-1 could be detrimental in some of these processes leading to the development of pathological events that may result in the phenotypic manifestations in Down syndrome individuals or in those with HC21 monogenic disorders.

Program Nr: 1004 from the 1999 ASHG Annual Meeting

Cloning and characterization of the mouse and rat homologs of *MEFV*, the gene for familial Mediterranean fever. *J.J. Chae[1], M. Centola[1], I. Aksentijevich[1], A. Dutra[2], M. Tran[3], K. Nagaraju[1], D.W. Kingma[3], P.P. Liu[2], D.L. Kastner[1]*. 1) National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, MD; 2) National Human Genome Research Institute, Bethesda, MD; 3) National Cancer Institute, Bethesda, MD.

Familial Mediterranean fever (FMF) is a recessive disorder characterized by episodes of fever with serositis or synovitis. Recently the FMF gene (*MEFV*) was cloned; the protein product, pyrin, belongs to a family of nuclear factors homologous to the Ro52 autoantigen, and is thought to be a regulator of inflammation in myeloid cells. In this study we cloned and characterized the mouse and rat homologs of *MEFV*. The murine gene contains ten exons with a coding sequence of 2304 bp, while the rat homolog has nine exons with a coding sequence of 2253 bp. A considerable amino acid sequence homology was observed between the mouse and human (47.6% identity and 65.5% similarity) and between the mouse and rat genes (73.5% identity and 82.1% similarity). The predicted rodent proteins have several important domains and signals, such as the B-box zinc finger domain, Robbins-Dingwall nuclear localization signals, and coiled-coil domain, found in human pyrin. However, probably because of an ancient frame-shift mutation, neither the mouse nor the rat protein has an intact C-terminal B30.2 domain, in which most of the FMF-associated mutations have been found in humans. Nevertheless, like the human gene, mouse *Mefv* is expressed in peripheral blood granulocytes but not lymphocytes. The mouse *Mefv* is localized on mouse chromosome 16 region A3-B1, thus extending a region of synteny with human chromosome 16p13.3 where *MEFV* is located. Overall similarities between mouse and human genes suggests that the generation of the mouse models should not only provide insight into the precise role that *MEFV* plays in inflammation but also create valuable tools for studying the molecular pathogenesis for FMF.

Program Nr: 1005 from the 1999 ASHG Annual Meeting

Fine mapping and genomic organization of human filamin genes FLN2 and FLN3. *C.F. Chakarova[1], P.F.M. van der Ven[2], D.O. Frst[2], M. Wehnert[1].* 1) Institute of Human Genetics, University of Greifswald, Germany; 2) Department of Cell Biology, University of Potsdam, Germany.

Filamins are a group of ubiquitous, dimeric actin cross-linking phosphoproteins of the peripheral cytoplasm who promote orthogonal branching of actin filaments and link them to the membrane glycoproteins. So far, three human filamin cDNA sequences - termed alpha, beta and gamma-filamin - were described and found to be highly conserved. The precise chromosomal position and the genomic organization, however, were described only for alpha-filamin (FLN1). In the present study, we mapped beta-filamin/FLN3 on chromosome 3 and gamma-filamin/FLN2 on chromosome 7 by FISH and PCR. High-resolution mapping was achieved by PCR on the GeneBridge 4 Human/Hamster Radiation Hybrid Mapping Panel (Research Genetics). This positioned the FLN2 gene 2.12 cR (0.57 cM) off D7S530 on 7q32 and the FLN3 gene on 3p14 between WI-3771 (1.31 cR; 0.35 cM) and WI-6691 (1.71 cR; 0.46 cM). Taking the established structure of the FLN1 gene and assuming that the genomic organization of the three known filamin genes could be very similar, a PCR-based primer walking approach was used to define the intron-exon boundaries of FLN2 and FLN3. In contrast to FLN1, FLN2 consists of 45 exons and 44 introns and FLN3 consists of 44 exons and 43 introns. All intron-exon boundaries follow the GT-AG rule. The average intron size of FLN1 and FLN2 genes is almost identical (404 bp and 433 bp, respectively). Introns were significantly larger in FLN3 (average size 1584 bp). Thus the estimated sizes of FLN2 and FLN3 are 27 kb and 80 kb, respectively. The majority of the intron positions are identical in all three filamin genes. Five introns are either missing or inserted in one or two of the paralogous genes. Exon 30 is deleted in the FLN2 gene leading to the loss of one "hinge" at the protein level. Instead, a novel exon (38A) is inserted, which should result in distinct functional properties of the resulting protein. In summary, our study provides evidence that the three filamin paralogs share a highly conserved genomic structure. The observed differences hint at a hitherto unknown functional diversity of filamin isoforms.

Program Nr: 1006 from the 1999 ASHG Annual Meeting

Mechanisms of transcriptional repression of the human *HPRT* promoter by DNA methylation. *C. Chen[1], M.C.K. Yang[2], T.P. Yang[1]*. 1) Dept. of Biochem. and Mol. Biology,; 2) Dept. of Statistics, University of Florida, Gainesville, FL.

The correlation between promoter hypermethylation and transcriptional repression is well documented, particularly among the X-linked housekeeping genes. The 5' region of the X-linked human hypoxanthine phosphoribosyl transferase gene (*hHPRT*) is unmethylated on the active allele but almost completely methylated on the inactive allele. *hHPRT* can be reactivated on the inactive X chromosome (X_i) by treatment with the DNA demethylating agent 5-aza-deoxycytidine (5aCdr), further implicating methylation in its transcriptional repression. To identify methylation sites that may be critical for maintaining repression of *hHPRT*, we have treated a human/hamster hybrid containing a single human X_i with 5aCdr and then examined by LMPCR the high resolution methylation pattern of the *hHPRT* 5' region in single-cell-derived clonal lines. Both spontaneous and 5aCdr-induced reactivation of *hHPRT* correlate with global demethylation of its 5' region. However, in 61 non-reactivated 5aCdr-treated clones examined, sporadic demethylation was observed at all but three CpG methylation sites, which always remained methylated. These three methylated CpG's may be critical for transcriptional silencing of *hHPRT* on the X_i. DNase I *in vivo* footprinting suggests that on the X_i, all three sites fall within a single phased nucleosome encompassing the major transcription initiation sites of *hHPRT*. However, only one site falls within a potential transcription factor binding site as defined by dimethyl sulfate and DNase I *in vivo* footprinting. 5aCdr re-treatment of those partially demethylated clonal lines that failed to reactivate *hHPRT* reveals no correlation between the reactivation frequency of *hHPRT* and the pre-existing level of demethylation, a finding consistent with the existence of critical methylation sites. Treatment with trichostatin A, a potent histone deacetylase inhibitor, fails to reactivate *hHPRT* in either of two hybrid cell lines containing a X_i suggesting that repression by methylation involves more than just histone deacetylase recruitment. We also present evidence of *de novo* methylation secondary to 5aCdr treatment.

Program Nr: 1007 from the 1999 ASHG Annual Meeting

Cloning and sequence analysis of two distinct cDNAs encoding cystic fibrosis transmembrane conductance regulator from the Atlantic salmon, Salmo salar. *J.M. Chen[1], C. Jacques[1], C. Cutler[2], B. Mercier[1], G. Boeuf[3], C. Ferec[1]*. 1) Centre de Biogenetique, Universite, Hopital, ETSBO, Brest, France; 2) Molecular Physiology Group, Bute Medical Buildings, St Andrews, UK; 3) Direction des Ressources Vivantes, IFREMER, Brest, France.

The Atlantic salmon, Salmo salar, is capable of adapting rapidly to transfer from freshwater to seawater. Investigation into this unique phenomenon of osmoregulation, which is believed to be a function of the human cystic fibrosis transmembrane conductance regulator (CFTR) homologue, would provide insights into the structure and function of the human CFTR gene. As a first step, we isolated the CFTR cDNA from mRNA samples prepared from salmon gill by routine RT-PCR using degenerated primers and rapid amplification of cDNA ends. DNA sequence analysis of the overlapping cloned PCR products revealed two clearly distinct cDNAs, designated as sCFTR-I and sCFTR-II respectively. The nucleotide sequence of sCFTR-I cDNA is 5496 bp long, encoding 1519 amino acids, and that of sCFTR-II is 5413 bp long, encoding 1518 amino acids. At the nucleotide level these two types of cDNA show an identity of 93.2% in the coding region and at the amino acid level, they are 95.3% identical. When compared to human CFTR, sCFTR-I, which turns out to be the most divergent form of CFTR characterised to date showes a 1% less identity than sCFTR-II both at the nucleotide level (60.3% vs 61.3%) and at the amino acid level (58.1% vs 59.1%). The two nucleotide-binding domains are the most highly conserved domains and the R domain is the least highly conserved one. Despite the high sequence homology in the coding region, the 110 bp nucleotides upstream from the translation start site is only 64.0% identical and the 3' untranslated region is 71.8% identical between the two sCFTR cDNAs, suggesting that they are to be encoded by two different genes. Future experiments are to investigate their expression pattern in different tissues, in different developmental stages, and functional analysis of these two sCFTR genes.

Program Nr: 1008 from the 1999 ASHG Annual Meeting

A rapid method for elucidating exon-intron boundaries and genomic structure of genes. *S. Cheng, S.D. Pandit.* Human Genetics, Genome Therapeutics Corporation, Waltham, MA.

We have developed a rapid method to elucidate the genomic structure of candidate genes. Previous methods for elucidating exon-intron boundaries have relied on subcloning of YACs/BACs and extensive screening of sub-libraries with cDNA probes. Other methods have utilized ligation mediated PCR (Xu, G., et al., (1998), Genomics, 47: 171-179) or Vectorette PCR strategies (Roberts, R. G., (1992), Genomics, 13: 942-950) for genomic structure elucidation. These protocols either involve extensive subcloning, or are technically challenging. The method described here utilizes PCR to identify exon boundaries in cDNA segments followed by direct sequencing of BAC clones to obtain full or partial intronic sequences. PCR is performed against both cDNA (cDNA clone or pools) and genomic DNA (BAC clone) templates. Comparing the PCR product size from cDNA and genomic DNA templates allows the identification of potential existing introns in the genomic region. When a particular PCR primer pair produces the expected size product from the cDNA template but no product or a larger product from the genomic template, this region presumably contains one or more introns that interrupt the PCR. We modified the 33P-cycle sequencing method and used the PCR primers to perform direct sequencing of BAC DNA to obtain high quality genomic sequence. Aligning the cDNA and genomic DNA sequences using "Sequencher" software package allowed us to define exon-intron boundaries. This simple and rapid method has proven useful for rapidly defining exon-intron boundaries which will prove useful in detecting variants in large number of candidate genes identified in positional cloning projects.

Program Nr: 1009 from the 1999 ASHG Annual Meeting

Cloning and genomic characterization of the human *PREB* gene; a conserved WD-repeat protein expressed in the mature adult and during human development. *C.T. Clelland[1], B. Levy[2], E. Nomoto[1], K. Hirschhorn[2], C. Bancroft[1]*. 1) Dept Physiology & Biophysics, Mount Sinai Medical Ctr, New York, NY; 2) Dept Human Genetics, Mount Sinai Medical Ctr, New York, NY.

PREB (prolactin regulatory element binding), a novel rat protein, exhibits sequence-specific DNA binding to site 1P of the prolactin promoter. This WD-repeat protein accumulates in the nucleus of rat pituitary cells and can transactivate the prolactin promoter *in vivo*. Furthermore, PREB can mediate both basal and PKA stimulated transcriptional responses in pituitary cells. The murine *Preb* gene exhibits a stage-specific expression pattern during embryogenesis, with high levels of mRNA transcripts observed in the developing pituitary gland, the hepatic system, and the cartilaginous condensations of the craniofacial and axial skeleton.

We have cloned the human *PREB* gene. The full length sequence of the 2.1kb human fetal brain cDNA has over 83% nucleotide identity with the rat *Preb* cDNA. In addition there is 89% identity and >91% similarity between the PREB protein homologues. The three putative WD-repeat motifs are highly conserved. Northern analysis of fifty adult tissues indicates that, although human *PREB* mRNA is ubiquitously expressed, levels of these transcripts vary considerably between tissue types, with the highest levels of expression in the pituitary gland, skeletal muscle, liver, salivary gland, placenta and pancreas. *PREB* is expressed in all human embryonic tissues examined. We have determined the intron/exon boundary structure of *PREB*, and have FISH mapped the gene to human chromosome 2p23, a region associated with a developmentally abnormal phenotype characterized as 2p trisomy syndrome. Further investigations into the role of this putative transcription factor may elucidate the role of *PREB* in human development.

Program Nr: 1010 from the 1999 ASHG Annual Meeting

PLAC1, an X-linked unique marker gene for placenta. *M. Cocchia, R. Huber, S. Pantano, M. Ko, D. Schlessinger.* Laboratory of Genetics, National Institute on Aging, NIH, Baltimore MD, 21224.

A novel human X-linked gene identified through genomic sequence analysis shows placental-specific expression, and has been named PLAC1. The gene maps 65 Kb telomeric to HPRT in Xq26 and has an ortholog in mouse that maps to the syntenic region of the murine X chromosome. The putative human and murine PLAC1 proteins are 60% identical and 77% homologous. A low but significant level of homology was also detected with ZP3 (Zona Pellucida 3). ZP3 is an important protein involved in the fertilization process in several species of eutherian mammals.

In order to understand how PLAC1 may be functioning in the developing placenta, we are studying the murine homolog expression pattern. Preliminary experiments using a PLAC1-GFP fusion protein suggest a cytosolic localization; and from *in situ* hybridization studies with the antisense mRNA during mouse embryogenesis, PLAC1 is expressed from 7.5 dpc to 13.5 dpc (days after coitus) in the primary trophoblastic giant cells and in the trophoblast cells; that is, in specialized extra-embryonic cells present only in eutherian mammals and critical for the processes of implantation and placentation. The hints from its homology to ZP3 and specific localization make PLAC1 a marker with a potential role in the establishment of the indispensable mother-fetal interface.

Program Nr: 1011 from the 1999 ASHG Annual Meeting

Characterization of the mouse *rotated abdomen* homolog, *Pomt1*. Mapping to chromosome 2 and exclusion of *Pomt1* mutations in *mdm*. *A. Cortes*[1], *P. García Pavía*[1], *L.A. Pérez Jurado*[1,2], *J. Cruces*[1]. 1) Dept. Bioquímica, Universidad Autónoma, Madrid, Spain; 2) Servicio de Genética, Hosp. Univ. La Paz, Madrid, Spain.

Rotated abdomen, *rt*, is a poorly viable recessive mutation of *Drosophila melanogaster* causing a clockwise twisted abdomen in affected individuals due to defects in embryonic muscle development. A human gene highly homologous to *rt*, *POMT1*, has been recently cloned that encode a protein with high homology to key yeast mannosyltransferases, PMTs. We have now characterized the mouse ortholog, *Pomt1*. A full length 3.1 kb *Pomt1* cDNA has been obtained by RT-PCR from various sources and encodes a 724 aa protein with 95%, 68%, and 54% similarities to human POMT1, *Drosophila* rt, and yeast PMTs, respectively. Northern blot reveals that *Pomt1* is ubiquitously expressed with higher levels in testis. Computer prediction of protein sorting suggests that the Pomt1 product could be located at the ER or Golgi membranes. Like the human homolog, the *Pomt1* gene consists of 20 exons. The *Pomt1* locus has been assigned in the EUCIB backcross to cM 22 of mouse chromosome 2, in a region of conserved synteny with human chromosome 9q34.1 where the *POMT1* locus maps. Interestingly, the *mdm* mutation (muscular dystrophy whit myositis), a mouse model of congenital muscular dystrophy, had been previously located at cM 26+/-4 of mouse chromosome 2. We have then screened mdm DNA for mutations in *Pomt1* by PCR amplification and sequencing of all exons and exon-intron junctions. No mutations were found, suggesting that *Pomt1* is not the site of the mutation responsible for the *mdm* defect.

Program Nr: 1012 from the 1999 ASHG Annual Meeting

Tight regulation of a mammalian promoter by bacterial *lac* operon elements. *C.A. Cronin, L. Abramova, L.A. Ligon, H. Scrable.* Dept Neuroscience, Univ Virginia, Charlottesville, VA.

Experimental control of gene expression is a powerful tool that can be used to elucidate aspects of gene function. Regulatable viral promoters have been used extensively to control target gene expression in both the *tet* and *lac* systems. The use of viral promoters, however, sacrifices the tissue specificity and level of expression that a gene's endogenous promoter would provide.

With the aim of controlling gene expression in the mouse, we introduced *lac* operator sequences into the promoter of the murine tyrosinase gene. A primary operator was introduced just downstream of the start of transcription by changing 25bp of the endogenous sequence. This maintained the endogenous spacing of promoter elements in the tightly spaced tyrosinase minimal promoter. Additional operator sequences were inserted 176bp and 526bp upstream from the primary operator. The final structure closely resembles that of the promoter of the bacterial *lac* operon.

Potentially regulatable tyrosinase promoters were cloned in front of the coding sequence of the b-galactosidase reporter gene. When VMM12 cells were transiently transfected with the *lac* repressor in combination with the reporter constructs, expression of the reporter gene was significantly reduced. When a lactose analog was added to the culture medium, expression of the reporter gene was restored to non-repressed levels. The repressed and induced levels of expression differed by an order of magnitude. To test how tightly expression could be controlled by these *lac* elements, the b-galactosidase coding sequence was replaced by the coding sequence for diphtheria toxin-A (DT-A). It has been estimated that only a few molecules of DT-A are necessary to poison a cell irreversibly. Repression of toxin expression in transfected VMM12 cells was tight enough to rescue the cell death seen in the unrepressed state.

Program Nr: 1013 from the 1999 ASHG Annual Meeting

Genomic Organisation of the PPP1CC gene located within the Noonan syndrome critical interval. *A.H. Crosby[1], M. Salehpour[1], R. Dong[1], K. Kalidas[1], A. Ion[1], S. Jeffery[1], M. Patton[1], H. Kremer[2], M. van Reen[2], I. van der Burgt[2], H. Brunner[2], E. Mariman[2], R. Kucherlapati[3], K. Montgomery[3]*. 1) Dept Medical Genetics, St George Hosp Med Sch, London, England; 2) Dept Human Genetics, University Hosp Nijmegen, Nijmegen, The Netherlands; 3) Dept Molecular Genetics, Albert Einstein College Medicine, NY, USA.

Noonan syndrome (NS) is an autosomal dominant developmental disorder characterised by typical facial dysmorphology, congenital heart defects and short stature. Whilst we have shown that this condition is genetically heterogeneous, we have previously localised one gene for NS to a 5cM interval on the long arm of chromosome 12 at band 12q24.1. More recent work has involved the fine mapping and characterisation of a number of both novel and previously described transcripts within the interval. One of these transcripts, the PPP1CC gene, is a serine-threonine protein phosphatase. Due to their involvement in a variety of cellular functions from muscle contraction through to cell cycle progression, the protein phosphatases constitute potential candidates for NS. In order to examine this gene for mutations, we have determined its genomic organisation. PPP1CC comprises 7 exons which span approximately 17.5kb of genomic DNA. This gene is now being evaluated for disease-specific mutations in our large cohort of NS patients.

Program Nr: 1014 from the 1999 ASHG Annual Meeting

Isolation of two *DAZAP* genes encoding proteins that interact with both DAZ and DAZL. *T. Dai[1], S. Tsui[1], E. Salido[2], S. Roettger[3], W. Schempp[3], P. Yen[1]*. 1) Medical Genetics, Harbor-UCLA Medical Center, Torrance, CA; 2) Pathology, University of La Laguna, Spain; 3) Human Genetics and Anthropology, University of Freiburg, Germany.

The *DAZ* (Deleted in AZoospermia) gene family on the human Y chromosome and the *DAZL* (DAZ-Like) gene on chromosome 3 encode RNA-binding proteins that are expressed only in germ cells. The *DAZ* gene cluster is deleted in about 10% infertile males with azoospermia, and *Dazl* knock-out mice are sterile, supporting a role for *DAZ* and *DAZL* in spermatogenesis. DAZ and DAZL differ in that DAZ contains 8-24 copies of a DAZ repeat, whereas DAZL contains only a single copy of the repeat. In addition, these proteins have different C-terminal sequences due to a frame-shift after the DAZ repeats. In order to compare the two proteins and elucidate their function, we used the DAZ protein as the bait in the yeast two-hybrid system and isolated two *DAZAP* (DAZ Associated Protein) genes. Interactions between DAZ and the DAZAP proteins were confirmed by *in vitro* binding assays, in which labeled DAZ bound to immobilized GST-fusion proteins of the DAZAPs. Similar binding assays showed that DAZL also interacted with the two DAZAP proteins. *DAZAP1* represents a novel gene encoding a 45 kd protein with two RNP motifs in the N-terminal region , similar to those found in hnRNP A/B and a *Drosophila* hnRNP protein hrp48. However, the C-terminal region of DAZAP1 is rich in proline instead of glycine. *DAZAP2* is identical to an anonymous gene, KIAA0058, previously isolated from a human male myeloblast cell line, KG-1. It encodes a 19 kd protein with no recognizable functional domains. *DAZAP1* is expressed most abundantly in the testis, whereas *DAZAP2* is ubiquitously expressed. *DAZAP1* and *DAZAP2* were mapped to 19p13.3 and 2q33-34, respectively, by FISH. The significance of the interactions between DAZ, DAZL and the DAZAPs remains to be determined.

Program Nr: 1015 from the 1999 ASHG Annual Meeting

Cloning of two genes from the 22q11 deletion syndrome proximal critical region. *S. Demczuk[1], A. Aurias[2], B. Dion[1]*. 1) Research Inst, PT 239, Montreal Children's Hosp, Montreal, PQ, Canada; 2) INSERM U509, Institut Curie, Paris.

Microdeletions of chromosome 22q11.2 are associated with a wide range of clinical abnormalities, the most frequent of which are conotruncal cardiac defects, thymus and parathyroids hypoplasia, a typical facial dysmorphism and cleft palate. A number of genes have been isolated from the 22q11 deleted region, but definitive evidence to incriminate one gene, rather than another in this pathology are missing.

We have chosen exon trapping as a method to build a transcription map of the proximal critical region for this syndrome. Two of the exons retrieved were identical in sequence to ESTs, strongly suggesting that they corresponded to genes.

Exon 39-2A2 is 97 bp and the corresponding EST (R88591) was completely sequenced and is 774 bp-long. The EST has been isolated from an adult brain library and recognizes a 2.6 kb transcript in adult heart, muscle, kidney, brain and lung RNAs. It maps a few kilobases telomeric to *DGCR6*. This EST has a strong homology to the Drosophila proline oxidase gene and is actually part of the recently cloned human proline dehydrogenase gene. It has been linked to the hyperprolinemia phenotype displayed by some 22q11 deletion syndrome patients.

The second exon, Po-1B9, is 104 bp-long and the analogous EST, H08046, isolated from an infant brain library is 1424 bp. Clone H08046 recognizes an approximately 3 kb transcript in adult pancreas RNA. It maps a few kilobases centromeric to the 5' of *DGCR5*. The EST was completely sequenced and database search revealed weak homology to the human diaphanous gene and to synapsin genes from a variety of species. Further characterization of this gene will be done in order to elucidate its potential function.

Program Nr: 1016 from the 1999 ASHG Annual Meeting

Structure and function of the 5' flanking region of human very long chain acyl-CoA dehydrogenase: possible bi-directional promoter activity. *J.H. Ding, G. He, B.Z. Yang, C.R. Roe.* Inst Metabolic Disease, Baylor Univ Medical Ctr, Dallas, TX.

Very long chain acyl-CoA dehydrogenase (VLCAD) catalyzes the initial beta-oxidation step of long chain fatty acids in mitochondria. Mutations identified in the coding region of the gene were shown to cause enzyme deficiency resulting in recurrent hypoglycemia and/or cardiomyopathy. It was postulated that down regulation of enzymes involved in fatty acid oxidation in the mitochondria was associated with the development of cardiomyopathy and heart failure. In order to understand the regulation of VLCAD at the transcriptional level, an inverse nested PCR-based method was used to clone the 1 kb fragment immediately 5' upstream of VLCAD. Sequence analysis of the fragment revealed many structural similarities with the promoter region of medium chain acyl-CoA dehydrogenase. In addition, sequence search in the GenBank database identified an unusual overlap of sequence covering the 5' upstream region of both VLCAD and Postsynaptic Density-95 (PSD95) genes. To investigate this unique structure and its role in regulating the transcription of both VLCAD and PSD95 genes, the overlapping fragment was cloned into a green fluorescence protein (GFP) reporter vector in both orientations. Transfection of these constructs and subsquent expression of GFP in human fibroblast cells demonstrated that the region contained regulatory elements essential for the promoter activity of both VLCAD and PSD95 genes. We believe that the understanding of VLCAD as well as PSD95 gene expression and regulation should provide insight into the development of cardiomyopathy and heart failure.

Program Nr: 1017 from the 1999 ASHG Annual Meeting

Cloning of KCNE-2 and KCNE-3: two new putative voltage-dependent K+ channel subunits. *A. Domenech, M.L. Arbones, X. Estivill, S. de la Luna.* Medical and Molecular Genetics, IRO, Barcelona, Spain.

To identify new genes in human chromosome 21, we have used an in silico approach. BlastN search of the ESTs database was performed with a public genomic sequence (GB acc. no. AP000052). A human EST (GB acc. no. AI246239) was considered for further study due to its similarity to KCNE-1. A corresponding cDNA was isolated from human adult heart cDNA, and its sequence determined, showing the presence of an open reading frame of 123 amino acids. We have named this new human gene as KCNE-2. To identify another putative voltage-dependent K+ channel subunits, a tBlastX search of human dbESTs was performed with the nucleotide sequence of KCNE-2. The consensus sequence of eigth overlapping ESTs showed the presence of an ORF of 103 amino acids. The cDNA was isolated from human adult colon cDNA. We have named this new human gene as KCNE-3. At the amino acid level, KCNE-2 and KCNE-3 have high similarity to human Isk protein (also known as minK), a membrane protein which induces slowly activating, voltage-dependent K+ channels when it is complexed to KvLQT1 (KCNQ-1). Mutations in either KCNE-1 or KCNQ-1 are responsible for the Jerwell and Lange-Nielsen syndrome, characterized by syncopal attacks and high risk of sudden death due to ventricular tachyarrythmia and severe bilateral deafness. Northern blot analyses with a KCNE-2 probe showed a 1.35 kb transcript highly expressed in human adult heart and a lower level of expression in skeletal muscle. A 3.5 kb transcript highly expressed in colon, small intestine, ovary and peripheral blood was detected with a KCNE-3 probe. KCNE-2 and KCNE-3 subcellular location has been studied by immunofluorescence analysis of COS-7 transfected cells. KCNE-2 and KCNE-3 are glycosilated proteins which localised to the plasmatic membrane being its N-terminus extracellularly located. Considering the high degree of similarity with Isk, the tissue specific expression pattern and the subcellular localisation, we suggest that KCNE-2 may participate in a cardiac voltage-dependent K+ channel, and KCNE-3 may participate in a colon voltage-dependent K+ channel.

Program Nr: 1018 from the 1999 ASHG Annual Meeting

Characterization of an enhancer region of the X-linked anhidrotic ectodermal dysplasia (EDA) gene. *M.C. Durmowicz, D. Schlessinger.* Laboratory of Genetics, National Inst on Aging, Baltimore, MD, 21224.

Because individuals affected with anhidrotic ectodermal dysplasia (EDA) have sparse hair, rudimentary teeth, and few eccrine sweat glands, the gene is believed to function at an early stage in ectodermal development, possibly at a branch point. Regulation of gene expression occurs at transcription and at mRNA splicing, producing eight transcripts that are differentially expressed at low levels during development. In ongoing studies of the regulation of transcription, a hierarchical promoter structure is emerging. Moving 5' from the coding region, one encounters a segment containing two active Sp1 sites that determine basal transcription; a binding site for LEF-1, a transcription factor already implicated in ectodermal formation; and a region, further described here, that shows enhancer activity. The enhancer region is centered approximately 610 bp upstream of the transcription start site. In transient transfection assays with an enhancer reporter construct, the 125 bp enhancer region is defined by its capacity to boost transcription from an SV40 promoter 4- to 5- fold in an orientation-independent manner. Deletion of 40 bp from either end of this segment abolishes enhancer activity. Oligos within each of these 40 bp regions detect binding proteins in mobility shift assays. Further analysis has detected three distinct binding sequences, with two of them similar to GATA and Nkx-2 sites, in the segment from nt-673 to nt-633, and a third site at the other end of the enhancer region, extending from nt-590 to nt-550 and not corresponding to any known class of enhancer-binding protein binding sites. Mutations in any of these sites abolish their activity in gel shift assays. Thus, there are at least three putative transcription factors that bind within two distinct enhancer regions of the EDA gene to activate transcription; the binding affinities are sufficient to permit affinity purification, now in progress.

Program Nr: 1019 from the 1999 ASHG Annual Meeting

Characterization of xnp-1, a *Caenorhabditis elegans* gene similar to the human XNP/ATR-X gene. *J.J. Ewbank*[1], *M. Fontes*[2], *L. Villard*[2]. 1) CIML - Campus de Luminy, Marseille Cedex9, France; 2) INSERM U491. Faculté de Médecine La Timone. 27 Bd Jean Moulin 13385 Marseille Cedex 5. France.

We have characterized a new *Caenorhabditis elegans* gene, that we have called xnp-1, which encodes the closest known non-mammalian relative of the human XNP/ATR-X protein. Mutations in the corresponding gene lead to several mental retardation syndromes in humans (ATR-X and Juberg-Marsidi syndromes). The nematode gene is composed of 10 exons and we have shown that a 4,3 kb transcript is produced from the xnp-1 locus. The 1359 residues XNP-1 protein is 33,6 % identical and 52,2 % similar to the human XNP/ATR-X protein. Such a degree of similarity over more than a thousand amino acid residues is likely to reflect a conserved cellular function for the two proteins. In two regions of more than 250 amino acids, the proteins display 70% identity. The human and the nematode proteins are putative DNA-helicases as they contain the seven characteristic domains of this family of proteins, which is involved in various cellular processes such as DNA repair, chromosome segregation, recombination or transcriptional regulation. In addition to the fact that similar proteins are encoded by the nematode of the human genes, they both have a partially identical genomic structure. These data may indicate that xnp-1 and XNP/ATR-X may have diverged from the same ancestral DNA-helicase gene and may have conserved identical functions at the cellular level. RNA interference and xnp-1 promotor-mediated GFP expression experiments currently being performed using the nematode gene will allow an exploration of the cellular mechanisms in which it is involved and give potential clues as to the role of its human counterpart.

Program Nr: 1020 from the 1999 ASHG Annual Meeting

LINE1 element tails: a possible role in alternative splicing relevant for primates genome evolution? *A. Ferlini[1,2], T. Patarnello[3], M. Dunckley[2], F. Muntoni[2].* 1) Istituto di Genetica Medica, Universita' di Ferrara, Ferrara, Italy; 2) Neuromuscular Unit, Imperial College School of Medicine, Hammersmith Campus, London, UK; 3) Dipartimento di Biologia, Universita' di Padova, Italy.

We further studied an already reported family with X-linked dilated cardiomyopathy caused by a dystrophin mutation involving a sequence originally classified as an Alu-like element. This sequence is however part of the unconventional 5 tail of a novel LINE1 element, named LIPMA2 and deposited in the Repbase (http://charon.girinst.org/). We defined the mutation as a 11Kb deletion in intron 11. This deletion brought the 5 tail of a LIPMA2, normally present in intron 11, close to a cryptic splice site, inducing the shuffling of part of the 5 tail into the dystrophin transcript. The pathogenic mechanism of this mutation provides the first experimental evidence that, in addition to the demonstrated 3' transduction model, a 5'transduction of tails co-mobilised with LINE1 elements can occur. Since this splicing mutation also had a tissue specific expression, this also suggests that LIPMA2 is involved not only in exon shuffling, but also in splicing regulation. In order to identify specific regions of the 5 tail involved in splicing regulation we are producing constructs containing different part of this tail to perform "in vitro" transcription/splicing assay studies.In order to investigate when this element appeared in the course of evolution, we tested by PCR and Southern blot analysis the Alu-like sequence in genomic DNA of several primate species as well as in other mammals and birds.Our results suggest that this sequence is primate specific. Sequence comparison of this part of the LIPMA2 5' tail in different primates revealed diagnostic mutations and the phylogenetic relationships were as expected. On this base, we can assume that the 5 LIPMA2 tail appeared at least 25-45 million years ago.In conclusion the mechanism of disease in this family with XLDC support the 5' transduction model and suggest that the 5 tail of LIPMA2 does indeed have a role in RNA remodelling. The primate specific nature of this sequence makes this hypothesis extremely intriguing for its evolutionary implications.

Program Nr: 1021 from the 1999 ASHG Annual Meeting

Modelling mutational spectra using recursive partitioning. *B.A. Fijal[1], R.M. Idury[2], J.S. Witte[1].* 1) Epidemiology and Biostatistics, Case Western Reserve University Cleveland, OH; 2) Axys Pharmaceuticals, La Jolla, CA.

A mutational spectrum is the pattern of changes that have taken place in a gene that is thought to cause a specific disease. While it is well known that the positions of hotspots and clusters of mutations within a spectrum indicate where functional domains and other areas of the protein that are essential for its proper functioning are located, very few statistical methods exist that satisfactorily identify both hotspots and clusters for this type of data. We present a method of analyzing mutational spectra that allows one to identify both hotspots and clusters. As an example we analyze a p53 mutational spectrum taken from colorectal cancer patients. The hotspots and clusters identified by our method and their possible relevance are compared to what is known of p53's structure and function. We show that information gained by modelling spectra with this method can compliment information on a protein's structure/function gained by other methods, such as searching for conserved regions across species.

Program Nr: 1022 from the 1999 ASHG Annual Meeting

The human *GTX* gene maps to 10q26, a region often deleted in gliomas. *J.Y. Garbern[1, 2], H.H.Q. Heng[2], R.-P. Wu[1, 2], C.J. Ye[3], R. Awatramani[1], J. Kamholz[1, 2].* 1) Dept Neurology, UHC 8B, Wayne State Univ, Detroit, MI; 2) Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI; 3) SeeDNA Biotech, Windsor, ON.

The murine *GTX* gene is an NKx-family homeobox gene that we have found is expressed in the central nervous system specifically in oligodendrocytes. The GTX protein is a sequence specific DNA binding protein and therefore is a potential regulator of gene expression in these cells.

We have now cloned and sequenced the human *GTX* gene from a brain cDNA library and a genomic library and mapped its chromosomal location by fluorescence in situ hybridization. The *GTX* gene is comprised of 3 exons separated by introns of 91 and 109 base pairs. The relative intron positions are identical between the human and murine genes, although the intron sizes differ between the species. The predicted human GTX protein is 97 % identical to its murine homolog across its 277 codon reading frame, with all variances lying outside of the homeodomain. A histidine at position 3 of the homeodomain is found in the NKx6.1 protein but is otherwise unique at that position among known homeodomain proteins, and may underlie its specific biological properties. We have mapped the human gene to 10q26.

Although the biological role(s) of GTX in the human brain have not been defined, its extremely high sequence similarity to the murine gene suggest common functions. The localization of *GTX* to a region of chromosome 10 that is often lost in gliomas raises the possibility that this gene may have tumor suppressor properties.

Supported in part by a Virtual discovery grant from the Karmanos Cancer Institute.

Program Nr: 1023 from the 1999 ASHG Annual Meeting

Genomic Structure and Localization of the IKBKAP gene to the Familial Dysautonomia Candidate Region on 9q31. *S.P. Gill[1], M. Leyne[1], J. Mull[1], C.B. Liebert[1], C.M. Robbins[2], H.W. Pinkett[2], I. Makalowska[2], C. Maayan[3], F.B. Axelrod[4], A. Blumenfeld[3], M. Brownstein[2], B.P. Chadwick[1], S.A. Slaugenhaupt[1]*. 1) Harvard Inst. Human Genetics, Massachusetts General Hospital, Boston, MA; 2) National Institute of Health, Bethesda, MD; 3) Hadassah University Hospital, Jerusalem, Israel; 4) New York University Medical Center, New York, NY.

Familial Dysautonomia (FD, Riley-Day Syndrome) is an autosomal recessive disorder characterized by the poor development and progressive degeneration of the sensory autonomic nervous system. The gene defect has a carrier frequency of 1:30 among people of Ashkenazi Jewish descent. Linkage studies have narrowed the candidate region for the gene (DYS) to a less than 0.5 cM area of chromosome 9q31. We have used positional cloning techniques to isolate transcripts from this region. Exon trapping identified 23 exons which were pooled and used to screen a cDNA library. Seven overlapping clones were isolated and sequenced to form 'CG-5', a 4390 base pair cDNA. Additionally, cDNA selection identified a clone which matched two groups of human ESTs through database searches. The sequences of these clones extended the 3' untranslated region of CG-5 to form a 6040 base pair cDNA. Database searches have shown this to be the IKappaBKinase(IKK)-complex Associated Protein (IKBKAP) gene. IKBKAP has been reported as an assembler and regulator of active kinase complexes and was originally mapped to 9q34. Southern and Northern analysis showed IKBKAP definitely maps to the Familial Dysautonomia candidate region in 9q31. We have determined the genomic structure of the gene and identified several single nucleotide polymorphisms and a single dinucleotide polymorphism during mutational screening of IKBKAP as a candidate gene for FD. No splicing or coding mutations have been found in the gene which are pathogenic in Familial Dysautonomia. However, until DYS is discovered, IKBKAP remains a candidate for FD, and may contain non-coding mutations.

Program Nr: 1024 from the 1999 ASHG Annual Meeting

Two functional copies of the *DGCR6*gene are present on 22q11 and lie within the low copy repeat, sc11.1. *R. Goldberg[1], L. Edelmann[1], E. Spiteri[1], R.K. Pandita[1], B. Funke[1], N. McCain[1], A. Skoultchi[2], R. Kucherlapati[1], B.E. Morrow[1]*. 1) Dept Molecular Genetics, Albert Einstein Col Medicine, Bronx, NY; 2) Dept of Cell Biology, Albert Einstein Col Medicine, Bronx, NY.

The *DGCR6*(DiGeorge Critical Region 6) gene encodes a putative protein with high sequence similarity to gonadal (gdl), a *Drosophila melanogaster*gene of unknown function (Demczuk et al., 1996). We mapped the *DGCR6*gene to chromosome 22q11 within a low copy repeat, sc11.1a, of which there are two members termed sc11.1a and b, originally identified by interphase FISH mapping with the cosmid, sc11.1. We identified a second copy of the gene, *DGCR6L*, within the sc11.1b locus. We sequenced genomic clones from both loci and determined that the putative initiator methionine is located further upstream than originally described in a position similar to the mouse and chicken homologs. *DGCR6L*encodes a highly homologous, functional copy with some base substitutions rendering amino acid changes. Expression studies of the two genes using multiple tissue cDNA panels indicates that both genes are widely expressed in fetal and adult tissues. Both sc11.1 repeats are deleted in most VCFS/DGS patients and map immediately adjacent to the low copy repeats, termed LCR22, that mediate the deletions associated with velo-cardio-facial syndrome/DiGeorge syndrome (VCFS/DGS). Interestingly, an intact HERVK retrovirus maps within sc11.1a, however, a second HERVK integration site is not present at the sc11.1b locus indicating that the integration of the retrovirus occurred after the sc11.1 duplication during evolution.

Program Nr: 1025 from the 1999 ASHG Annual Meeting

Identification and expression analysis of C21orf5, the ortholog of which leads to embryonic lethality in C. elegans using double-stranded RNA-mediated interference. *M. Guipponi[1], K. Brunschwig[2], Z. Chamoun[3], J. Kudoh[4], HS. Scott[1], S. Al Samadi[3], AL. Delezoide[5], Z. Chettouh[3], C. Rossier[1], N. Shimizu[4], F. Mueller[2], JM. Delabar[3], SE. Antonarakis[1].* 1) Div Med Genet, Geneva, Switzerland; 2) Inst Zool, Fribourg, Switzerland; 3) CNRS URA 1335, Paris, France; 4) Dept Mol Biol, Keio Univ, Sch Med, Tokyo, Japan; 5) Hop. Necker, Hop. Robert Debre, Paris, France.

Human chromosome 21 has been intensively studied, since trisomy 21 causes Down syndrome (DS). Molecular analyses of patients with partial trisomy 21 allowed the characterization of the Down Syndrome Critical Region (DSCR). To isolate genes from this region on 21q22.2, a pool of cosmids positive for D21S333/S334 was selected for cDNA selection. From the 27 transcription units identified, one, CER5-0, was found to be identical to several HC21 trapped exons. cDNA library screening and RACE were used to determine the cDNA sequence of 7288 bp with an ORF of 2298 aa, termed C21orf5 (chromosome 21 open reading frame 5). Analysis of the 21q22.2 genomic DNA sequence revealed that C21orf5 consists of 37 exons that extend over 130kb and maps between CBR and D21S334. Northern blot analyses revealed a major ubiquitously expressed transcript of 8.5kb. RNA in situ hybridization on human embryos revealed a weak and ubiquitous signal with strong labeling only in restricted areas. In the CNS, strong labeling might correspond to neuronal cells which have already migrated and which have started to differentiate. C21orf5 shared significant homology with the E. nidulans DopA protein, a potential novel component of tyrosine kinase signal transduction pathway and with D. melanogaster, S. cerevisiae and C. elegans predicted protein from the different genome sequencing projects. Double-stranded RNA-mediated interference in C. elegans were performed by injection of dsRNA derived from the ortholog gene in C.elegans into the gonads of adult wild-type worms. The major phenotype observed in the progeny generated by the injected animals was embryonic lethality. These results indicated that C21orf5 is essential for the survival of C. elegans, and may be involved in one of the DS phenotypes.

Program Nr: 1026 from the 1999 ASHG Annual Meeting

Functional characterization of the *PMP22* gene promoters. *M. Hai[1], S.I. Bidichandani[2], P.I. Patel[1,2].* 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Neurology, Baylor College of Medicine, Houston, TX.

Overexpression of peripheral myelin protein-22 (PMP22) results in Charcot-Marie-Tooth Type 1A, one of the most common inherited peripheral neuropathies, while underexpression of PMP22 results in hereditary neuropathy with liability to pressure palsies. The dosage of PMP22 is therefore critical, and transcriptional control could be a mechanism of ensuring correct levels of gene expression. *PMP22* expression is driven by two alternative promoters (P1 and P2), that give rise to two different transcripts.The transcript from the P1 promoter is strongly associated with myelination by Schwann cells.Transient transfection of a deletion construct series of the P1 and P2 promoters into fibroblast and Schwann cell lines had provided preliminary evidence for the existence of transcriptional regulatory elements. Electrophoretic mobility shift assays (EMSA) detected regulatory factors that bind to these elements. Candidate regulatory regions of the P1 promoter were assayed for regulation of a heterologous promoter, in different Schwann cell and other cell type lines. A 43-bp region of the P1 promoter was able to regulate a heterologous promoter in a cell-type specific manner. EMSA competition experiments were used to localize the binding site of the putative regulatory factor. Mutation analysis of this 43-bp region has been designed to further confirm its role in the regulation of the *PMP22* gene. In order to develop a method of reducing expression of the *PMP22* gene, triplex forming oligonucleotides (TFOs) were developed that bind to regions of the P1 and P2 promoters. TFOs form triplex DNA with purine-rich regions, and reduce gene expression by interfering with the transcription factors. High-affinity TFOs binding to the P1 promoter competed with the binding of a protein factor, and may be good candidates for regulatory agents. The P2 promoter TFOs bind with high affinity to their target region, but are not able to compete with the simultaneous binding of a protein factor. Together, these two lines of study will further our understanding of transcriptional control of the *PMP22* gene.

Program Nr: 1027 from the 1999 ASHG Annual Meeting

_IS_-RT-PCR localisation of MMP gene expression in human breast biopsy material. *L.M. Haupt[1], M.G. Irving[2], R. Irving[1], L.R. Griffiths[1]*. 1) Genomics Research Centre, Griffith University Gold Coast, Australia; 2) Victoria University, Wellington, New Zealand.

The in situ-reverse transcription-polymerase chain reaction (IS-RT-PCR) was successfully adapted from a previously reported in vitro assay (1), for use in archival paraffin embedded human breast biopsy material. IS-RT-PCR allows the site of amplification and hence gene expression to be localised in situ, and was utilised to examine gene expression of several members of the matrix metalloproteinase (MMP) family. MT1-MMP (membrane-type1-MMP), the activator of MMP-2, MMP-1 (interstitial collagenase) and MMP-3 (stromelysin-1) were examined in progressive breast biopsy lesions from poorly invasive breast carcinomas to lymph node metastases. Critical to the success of the IS-RT-PCR technique in paraffin embedded tissue are primer design and specificity, along with protocol steps including tissue fixation, protease digestion, and the use of sufficient inter-assay negative and positive controls. Validation of gene expression was confirmed through the use of two negative controls, an RNase/DNase control and a no reverse transcription (RT) control. The ubiquitous gene beta-actin served as a positive control for gene expression, with tissue morphological integrity confirmed by the haematoxylin/eosin (H&E) stain. Through utilisation of the IS-RT-PCR technique, the three MMP genes examined were able to be loclised to both the epithelial tumour and stromal components within the paraffin embedded tissue. The expression patterns observed confirmed both the previously observed associations of MMP genes with breast carcinoma and the specificity of IS-RT-PCR. Results indicate distinctive roles for MT1-MMP, MMP-1 and MMP-3 throughout the progression of breast neoplasia toward lymph node metastasis. 1. Haupt LM, Thompson EW, Griffiths LR, Irving MG (1996), Biochem Mol Biol Int 39:3:553-561. b.

Program Nr: 1028 from the 1999 ASHG Annual Meeting

An imprinted antisense transcript at the human *GNAS1* locus. *B.E. Hayward, D.T. Bonthron.* Human Genetics Unit, Univ Edinburgh Western Gen Hos, Edinburgh, Scotland.

The upstream region of the *GNAS1* gene contains two oppositely imprinted first exons, which encode two unrelated polypeptides and are separated by only 11kb of genomic sequence. The 5-most of these, encoding NESP55, is expressed from the maternal chromosome and methylated on the paternal allele; conversely the other exon, encoding XLas, is expressed from the paternal chromosome and methylated on the maternal one. The differentially methylated CpG-rich region associated with the XLas exon extends approximately 3kb upstream of its putative initiation codon and here we describe another transcript originating in this region. This transcript, which has an orientation antisense to XLas, has multiple alternative splice forms and terminates approximately 20kb 5 to the NESP55 exon. Although this RNA lacks coding potential, its sequence is very highly conserved. Nonetheless, the identification of one rare single-nucleotide polymorphism allowed us to establish that this antisense transcript is imprinted. Furthermore, this transcript is not detected by RT-PCR using RNA from the parthenogenetic lymphoblastoid cell line FD, although it is present in normal female lymphoblastoid cell RNA. Antisense transcripts have been described for other imprinted genes and suggested to play a role in regulating in cis the coding (sense) transcripts. Traversing as it does the maternally expressed NESP55 promoter, the paternally expressed *GNAS1* antisense transcript could play a major role in the regulation of this complex gene.

Program Nr: 1029 from the 1999 ASHG Annual Meeting

Identification of a novel gene expressed in human bone marrow stromal cells. *N. Ho, L. Jia, L. King, C. Driscoll, E. Gutter, M. Anderson, C. Francomano.* Medical Genetics Branch, NHGRI/NIH, Bethesda, MD.

To identify bone and cartilage-specific genes, to explore their functions and to study their relationship with normal skeletal growth and development as well as dysplasias, a project termed Skeletal Genome Anatomy Project SGAP (Jia et. al, 1997) involving cataloguing genes expressed in bone and cartilage and developing a tissue bank comprising bone, cartilage, tendon, synovium and ligament has been established. By high-throughput sequencing analysis from both human bone marrow stromal cells and normal human trabecular bone cells, 8666 ESTs were isolated. More than 100 ESTs with novel sequences, representing genes that are as yet uncharacterized, were identified. The clones containing the novel ESTs fragments were then sequenced and characterized.

One cDNA clone derived from bone marrow stromal cells carried an insert with novel human sequence of 4.7 kb in size. This gene is localized to the pericentromeric region (10p11.1-10q11.1) of chromosome 10 by radiation hybrid mapping using the Stanford 3 panel.Reaffirmation of the chromosome location by fluorescent-in-situ hybridization is in progress.By sequencing and BLAST searching against NCBI GenBank database, this 4.7 kb sequence shows no homology to any known gene. Part of the domain of the gene has a 63% amino acid identity to glutamate permease. To determine the expression pattern of this gene in different tissues, a probe containing 1.2 kb sequence of the gene is now being constructed and hybridized against multiple human tissue mRNA.

In conclusion, further characterization of novel genes expressed in human bone marrow stroma and trabecular bone will facilitate greater understanding into the pathophysiology of normal and abnormal processes of skeletal development.

Program Nr: 1030 from the 1999 ASHG Annual Meeting

Persistence of Foreign DNA in Mice after Oral or Parenteral Application. *U. Hohlweg[1], S. Dumrese[1], R. Schubbert[2], W. Doerfler[1].* 1) Institute for Genetics, Univ. Cologne, Cologne, NRW, Germany; 2) Medigenomix GmbH, Mnchen, Germany.

In previous work, we have demonstrated that orally administered foreign DNA as bacteriophage M13 DNA or plasmid pEGFP DNA with no homology to mouse DNA can persist in fragmented form in the gastrointestinal (GI) tract. These DNA fragments can penetrate the intestinal wall and reach the nuclei of leukocytes, spleen and liver cells. There is evidence that in spleen cells this foreign DNA can become covalently linked to genomic DNA with a high degree of homology to mouse DNA. We have now extended these studies to DNA which is enclosed in cells. To mimic the natural exposure of DNA to the GI-tract, we have fed mice with soybean leaves. From all segments of the gut, the complete small subunit of the ribulose-1,5-bisphosphate (RuBP) carboxylase gene could be retrieved and characterized by Southern blot hybridization analyses. These results suggest that DNA within cells is protected against the attack by nucleases and might have a better chance to penetrate the intestinal wall than naked plasmid DNA. Maximal fragment lengths of 337 bp from the RuBP carboxylase gene can be traced by PCR in some of the liver and spleen samples. The medical and evolutionary implications of these findings may be considerable. More recently, we have analyzed the fate of DNA (pRSVGFP) in mice upon intramuscular (i.m.) injection. The injected DNA (5 mg) can be retrieved by PCR (772 bp fragment length) in DNA from the injected muscle at least up to 4 weeks after injection and up to 24 h in DNA from organs remote from the injection site, like blood, liver and contralateral muscle. In order to investigate the uptake and expression of the injected DNA, we have analyzed the injected muscle and other organs for the expression of the green fluorescent protein (GFP) by fluorescence microscopy. Furthermore, the injected DNA with a length of 593 bp can be discovered by PCR in the contents of different segments of the intestine. These findings suggest that foreign DNA injected i.m. is not completely degraded in the organism and is eliminated via the liver-bile-intestinal pathway. (This research was supported by the Federal Ministry of Education and Research - BMBW - BEO 0311110).

Program Nr: 1031 from the 1999 ASHG Annual Meeting

Identification of Dyxin: A Novel Intracellular Binding Partner of a-**Dystroglycan.** *K.H. Holt, R.H. Crosbie, K.P. Campbell.* Howard Hughes Medical Institute, Dept. of Physiology & Biophysics and Dept. of Neurology, Univ. of Iowa College of Medicine, Iowa City, IA.

Dystroglycan functions as a component of the dystrophin-glycoprotein complex (DGC), to link the extracellular matrix via the a-dystroglycan-laminin-2 interaction, with the intracellular cytoskeleton via the b-dystroglycan-dystrophin interaction. The DGC stabilizes the muscle fiber against the constant forces of contraction. Although mutations in dystroglycan have yet to be identified in humans, mutations in various subunits of the DGC destabilize a- and b-dystroglycan at the sarcolemma. Furthermore, targeted deletion of the dystroglycan gene results in an early embryonic lethality in mice. In an attempt to learn more about the role of dystroglycan in muscle, we performed a yeast two-hybrid screen to search for novel intracellular binding partners of b-dystroglycan. We reasoned that this approach might identify proteins which interact transiently with b-dystroglycan, compared to integral components of the DGC, which for the most part have been discovered by biochemical purfication and cloning. We report here the identification of a novel human b-dystroglycan-binding partner, termed Dyxin, which contains two tandem LIM domains and is highly expressed in striated muscle in humans.

Program Nr: 1032 from the 1999 ASHG Annual Meeting

Characterization of the Lafora's Progressive Myoclonus Epilepsy (EPM2A) gene and protein. *L. Ianzano[1], B.A. Minassian[1], A.V. Delgado-Escuieta[2], G.A. Rouleau[3,4], S.W. Scherer[1].* 1) Genetics, Hospital for Sick Children, Toronto, Ontario, Canada; 2) Dept. of Neurology,UCLA School of Medicine and DVA West LA DVA, West Los Angeles, CA; 3) Dept. of Human Genetics, McGill University, Montreal, Quebec, Canada; 4) Centre for Research in Neuroscience, Montreal General Hospital, Montreal, Quebec, Canada.

Lafora's disease is an autosomal recessive form of adolescent progressive myoclonus epilepsy (PME) characterized by myoclonus, decreasing mental function, and death within 10 years of onset. The definitive diagnosis of Lafora's disease is based on detection of characteristic periodic acid Schiff-positive polyglucosan inclusions (Lafora bodies). In 1998, using a positional cloning approach, was been identified a gene (EPM2A) at chromosome 6q24 that causes LD. In the course of this study it was determined that genetic hetereogeneity existed indicating another gene elsewhere in the genome can cause the same phenotype. Moreover, the gene encodes a protein (laforin) of 332 amino acids with a consensus amino acid sequence indicative of a protein tyrosine phosphatase (PTP). To understand the role of the protein in the pathology of the disease we have raised antibodies to two different 'unique' portions of the peptide and are studying its expression at the tissue and cellular level. We have so far identified 10 mutations in 16 families. Of those, 5 are nonesense or deletion mutations and the other 5 are missense mutations. Work is underway to identify more mutations and to study the effect of missense mutations on the structure and function of laforin. Moreover, we have initiated experiments to generate a knock-out mouse; model with germ line inactivation of EPM2A by homologous recombination in ES cells. Two BACs were identified containing the complete EPM2A murine gene. The genomic structure of the mouse gene was determined. Using a segment from the 5' region a targetting vector was constructed that upon proper integration would replace EPM2A exon 1 with a neomycin-resistance gene cassette. Experiments are now underway to electorporate the construct into R1 ES cells. Homologous recombinants will be screened by Southern blot-hybridization analysis.

Program Nr: 1033 from the 1999 ASHG Annual Meeting

The gene structure of *MJD*. *Y. Ichikawa[1], M. Hattori[2], A. Toyoda[2], K. Ishii[2], S.-Y. Jeong[3], H. Hashida[1,3], N. Masuda[1,3], K. Ogata[1], F. Kasai[4], M. Hirai[4], J. Goto[1,3], G.A. Rouleau[5], Y. Sakaki[2], I. Kanazawa[1,3].* 1) Dept. of Neurology, Univ. of Tokyo, Tokyo, Japan; 2) RIKEN Genomic Science Center, Kanagawa, Japan; 3) CREST, Japan Sci. and Tech. Corp., Tokyo, Japan; 4) Dept. of Biological Sciences, Univ. of Tokyo, Tokyo, Japan; 5) Dept. of Neurology, McGill Univ. Montreal, Quebec, Canada.

Machado-Joseph disease (MJD) is an autosomal dominant neurodegenerative disorder characterized by cerebellar ataxia and variable associated symptoms. The disease is caused by an unstable expansion of the CAG repeat in *MJD* that maps to chromosome 14q32.1. The original cDNA clone, MJD1a, consists of 1776bp and four exons. We present here the update results of the gene structure of *MJD*. Northern blot analysis showed that the size of mRNA is approximately 7.8kb which is larger than MJD1a. To obtain a cDNA clone for *MJD*, we screened four human cDNA libraries; whole brain, caudate, retina, and testis. Twenty seven clones were obtained, including 21 independent clones. Most of the clones have a new exon which we have previously reported. Two clones (#H4-1. #H13-2) have longer 3'UTR region than the one that has been reported. Until now, we identified two alternative splicing sites and four polyadenylation signals. Therefore, there are at least 6 transcription products of *MJD*. To determine the genomic structure, we obtained 13 cosmid clones from a human 14 chromosome library and four BAC clones from a human genomic BAC library. FISH analyses was performed to determine the chromosomal localization of these clones. Eight cosmid clones and all BAC clones were mapped on 14q32.1, and the other cosmids were localized on 14q21-22. Using PCR and fiber FISH analyses, we made a contig of 6 cosmid clones and a BAC clone which were localized on 14q32.1 The genome sequencing of *MJD* is currently underway using nested deletion method. We sequenced approximately 75kb of the contig and identified three new introns. Taken together, *MJD* is composed of at least 8 exons. The gene structure of *MJD* appears to be complicated than the one that has been first reported.

Program Nr: 1034 from the 1999 ASHG Annual Meeting

Identification of a splicing enhancer sequence within exons of the dystrophin gene using a chimeric dsx pre-mRNA. *T. Ito[1], Y. Takeshima[2], H. Sakamoto[3], H. Nakamura[2], M. Matsuo[1]*. 1) Division of Genetics, ICMR, Kobe Univ. School of medicine, Kobe, Hyogo, Japan; 2) Pediatrics, Kobe Univ. School of Medicine, Kobe, Hyogo, Japan; 3) Biology, Kobe Univ. Faculty of Science, Kobe, Hyogo, Japan.

Pre-mRNA splicing is an essential process in the expression of most eukaryotic protein-coding genes. Several factors comprise the splicing mechanism thus making it complicated. The splicing enhancer sequence (SES) is one of the cis-acting elements located within an exon sequence. It consists of a polypurine-rich stretch and enhances the splicing of upstream introns. The SES in exon19 of the dystrophin gene has been reported earlier. We,thus,investigated SES candidate regions between exons 40 and 60. The candidate SES region was determined by selecting the longest purine segment that does not include a T nucleotide and also,the same segment must include as many AAg repeats possible. Using this criteria, 3 candidate SES regions in exons 43,46,and 53 were selected. SES was examined in vitro by analyzing the splicing of heterogous introns of the doublesex gene pre-mRNA in HeLa cell nuclear extracts. In this in vitro system,the splicing of chimeric dsx pre-mRNA is dependent on the downstream sequence. To evaluate SES, the template plasmids for chimeric dsx pre-mRNA was cut with restriction enzymes and various artificial oligonucleotides were inserted in the recognition sites. The reaction products were then electrophoresed and quantified. An artificial oligonucleotide,mimicking SES in exons 43 and 53,enhanced active splicing of an upstream intron in this chimeric pre-mRNA while the SES in exon 46 was noted to possess weak splicing enhancer activity. Among the 3 selected SES regions,the SES in exon 43 was recognized as having the strongest splicing enhancer activity. We have identified novel splicing enhancer sequences in exons 43 and 53 of the dystrophin gene.These results of in vitro splicing experiments showed that some of exons of the dystrophin gene have splicing enhancer sequence within it.

Program Nr: 1035 from the 1999 ASHG Annual Meeting

Identification of new mouse ABC Transporter gene related to ABCP, Mapping to chromosome 5. *P.K. Jain[1], V. Koneti Rao[2], T. Fojo[2], S. Bates[2], M. Dean[1]*. 1) NCI-FCRDC, NIH, Frederick,MD; 2) NCI-DCS, NIH.

ATP binding cassette (ABC) transporters, or traffic ATPases comprise a superfamily of proteins that couple the hydrolysis of ATP to the translation of solutes across a biological membrane. These transporters are wide spread among all living organisms. They accomplish not only the uptake of nutrients in bacteria, but are involved in diverse cellular processes. Moreover, some hereditary human diseases are caused by defects in the ABC transport system such as Cystic Fibrosis, Adrenoleukodystrophy, Age-related Macular Degeneration, Stargarts disease, Dubin Johnson syndrome, and Sideroblastic anemia. Although of major significance, details of the molecular mechanism by which these systems exert their functions are still poorly understood. Within the ATP binding domain, the second motif, walker B, is immediately preceded by a highly conserved sequence motif, known as the linker peptide. This sequence has proven to be a useful tool in identifying putative family members. By these criteria, a few proteins have also been recognized as a typical ABC protein that clearly serve functions other than transport. A recently found novel human ABC protein, ABC 50, contains no transmembrane domains. We have identified a new mouse gene by employing BLAST search against a conserved sequence from TM domain. An EST AA 277174 was identified which did not have any sequence identity with known ABC genes. The mouse BAC clone was identified by PCR screening, using the primers from 3 sequence of the EST. The sequence data of the BAC clone confirmed the sequence of the EST. Additional sequencing of the clone showed similarity with other mouse and rat ESTs and clones. The contig was assembled; the clone was sequenced further, and intron/exon boundaries were identified. The FISH analysis showed signal on mouse chromosome 5, using the BAC clone as a probe on a normal metaphase spread. Further FISH experiments on a normal human metaphase spread identified a syntenic region on chromosome 8p. Subsequently we are attempting to identify the human homologue. These results will help in analyzing the disorders mapped in this syntenic region.

Program Nr: 1036 from the 1999 ASHG Annual Meeting

A DNA architecture prone to spontaneous mutagenesis in the ITGB genes may have contributed to the expansion of the integrins network of adhesion molecules. *T. Jamal, K. Dellagi, D.M. Fathallah.* Dept Molecular Genetics, Institut Pasteur de Tunis, Tunis, Tunisia.

The human integrins network mediates a wide range of essential adhesive functions. It is formed by the association between 8 b and 14 a subunits. The diversity of the repertoire of integrins mediated-interactions is based upon the various combinations between the two subunits. To investigate the contribution of spontaneous mutagenesis in the generation of integrins functional versatility, we have first analyzed in the ITGB 2, 3 and 4 genes , the regions affected by mutations causing three integrins-related genetic disorders respectively: Leukocyte Adhesion Deficiency, Glanzmann Thrombasthenia and Epidermolysis Bullosa with Pyloric Atresia, for specific sequences prone to spontaneous mutations. Single base substitutions occurred mostly in the CpG dinucleotide mutation hot spot or affected dinucleotides GG,GC and CC that have high relative mutability. Most of the deletions were associated with consensus sequences known to be prone to this type of DNA alteration. Some of the deletions observed occured through the deletional mechanism mediated by the presence of palindromic or quasipalindromic sequence. The study was extended to the eight b integrins coding sequences, to show that the DNA architecture of the b integrins coding sequences is characterized by the presence of high copy numbers of DNA motifs that are prone to spontaneous mutations such as palindromic sequences, the putative polymerases a and b arrest sites or the dinucleotides CG,GC,GG and CC. We have also observed that codon usage in the human b integrins genes does not fully comply with the rule of CG containing supression codons observed in other human or vertebrates genes. Furthermore, use of the mutability prediction software: MUTPRED showed that the human b integrins genes have high overall mutability coefficients and defined the codons that have elevated mutability indexes. We concluded that the occurrence of spontaneous mutations favored by the integrins genes specific DNA architecture may have contributed to the genesis of the various a/b associations and hence to the expansion of the integrins repertoire.

Program Nr: 1037 from the 1999 ASHG Annual Meeting

Human CCTb1 and CCTb2, two novel isoforms of Phosphocholine Cytidylyltransferase: Characterization of the cDNAs and Mapping of the Gene Locus to Human and Mouse Chromosome X. *M.A. Karim[1], A. Lykidis[1], S. Jackowski[1,2]*. 1) Dept of Biochemistry, St Jude Children's Res Hosp, Memphis, TN; 2) Dept of Biochemistry, University of Memphis, Memphis, TN.

CTP:phosphocholine cytidylyltransferase (CCT) is a key regulator of phosphatidylcholine biosynthesis. A single isoform, CCTa, has been studied extensively. We have recently identified two additional isoforms termed CCTb1 and CCTb2. Human CCTb1 and CCTb2 encode 330 and 369 amino acid proteins, respectively, that differ at their carboxy termini and arise from alternate splicing of the same gene. CCTb1 and CCTb2 mRNAs were expressed differentially in multiple human tissues, whereas CCTa was uniformly expressed as determined by Northern blot and RT-PCR. Both isoforms of CCTb were expressed in brain, with CCTb2 being dominant. Liver also expressed both isoforms, but CCTb1 gave a stronger signal. Placental tissue contained CCTb1 transcripts with no detectable signal for CCTb2. On the other hand, CCTb2 was the predominant isoform expressed in HeLa cells whereas a lower amount of CCTb1 was detected. Both CCTa and both CCTb isoforms were expressed in fetal lung whereas mRNA from adult lung did not yield a signal for CCTb and only CCTa was expressed. The novel gene encoding CCTb, named PCYT1B, was mapped by fluorescence in situ hybridization (FISH) to human chromosome Xp22 using a BAC containing human genomic DNA as a probe. The mouse CCTb gene also mapped to the conserved syntenic locus of mouse chromosome XC3 as determined by FISH using a 13 kb CCTb genomic clone from a l-phage mouse genomic library. In contrast, CCTa is encoded by PCYT1A located on mouse chromosome 16. The PCYT1B gene structure is currently being determined.

Program Nr: 1038 from the 1999 ASHG Annual Meeting

Illegitimate Splicing of 5 of 7 Investigated in-frame Exons of the Neurofibromatosis Type 1 Gene. *D. Kaufmann[1], W. Leistner[2], S. Hoffmeyer[3], W. Just[4], P. Kruse[1], B. Bartelt[1]*. 1) Department of Human Genetics , University of Ulm, Ulm, Germany; 2) Max Delbrueck Centre for Molecular Medicine, Berlin, Germany; 3) Epidauros AG, Bernried, Germany; 4) Department of Medical Genetics, University of Ulm, Ulm, Germany.

Mutations in the regulatory sequences of human genes necessary for splicing can lead to skipping of exons. Such a mutation was found recently in cells of a Neurofibromatosis type 1 (NF1) patient leading to the skip of exon 8 of the NF1 gene. Investigations on human cell lines, primary cell cultures and cells in vivo revealed that this in-frame exon was also skipped in all cells examined at a low frequency (about 0.6 %). It is not due to mutations in single cells as shown in clones of HeLa cells. Of seven in-frame exons of the NF1 gene investigated afterwards illegitimate exon skipping was found for exon 7, 9 and 10b at a low percentage but not for the exons 10a and 10c. The alternative exon 9br, expressed normally only in brain, was inserted at a low percentage in all cell types tested. Transfection of HeLa cells with hnRNP-A1 or SF2/ASF, two proteins belonging to the splice factors, does not influence the occurrence of this illegitimate splicing. According to simulations of the two-dimensional minimum-free-energy structures of hnRNA illegitimately spliced or not, we propose that the illegitimate splicing of in-frame exons is due to the rare occurrence of metastable structures of hnRNA after transcription. These would lead to misfolded splice donor site sequences which are not recognized by the splice machinery. We speculate that illegitimate splicing limits the maximal number of in-frame exons in human genes.

Program Nr: 1039 from the 1999 ASHG Annual Meeting

Cloning and genomic structure of a 9.5-kb transcription factor-like nuclear regulator (TFNR), closely localized to the survival motor neuron gene. *A.R. Kelter[1], J. Herchenbach[1], F. v. Deimling[1], T. Liehr[2], J.M. Scharf[3], L.M. Kunkel[3,4], W.F. Dietrich[4], B. Wirth[1].* 1) Inst. Human Genetics, Bonn, Germany; 2) Inst. Human Genetics and Anthropology, Jena, Germany; 3) Children's Hospital, Boston, MA, USA; 4) Harvard Medical School, Boston , MA, USA.

Spinal muscular atrophy (SMA) is caused by homozygous mutations in the survival motor neuron gene (SMN1). However, rare familiar and sporadic cases, so called atypical SMA forms have been described, where SMA patients show additional atypical features, such as axonal neuropathies, brain atrophy with homozygous absence of SMN1 and usually with larger deletions within 5q13. We postulated that atypical SMA forms might represent contiguous gene syndromes caused by additional deletions/mutations of genes adjacent to SMN1. The SMA region contains a 500-kb duplication and inversion including the H4F5, SMN, NAIP and BTF2p44 gene copies. The region between the H4F5 copies is still unknown.

We describe a novel gene, called TFNR (for transcription factor-like nuclear regulator), that maps close to H4F5/SMN gene copies. Fluorescence in situ hybridization analysis with a BAC that covers the TFNR gene shows a strong signal on 5q13 and a weaker signal on 5p14-p15.1. TFNR shows ubiquitous expression of a 9.5 kb transcript with the strongest expression in cerebellum and a second small transcript at 1.9 kb. TFNR encodes a protein of 2190 amino acids (aa) and a molecular weight of 245 kD of unknown function . The coding region is organized in 30 exons and contains within exon 16 a 9x repeated 55-aa fragment. The putative aa-sequence is highly hydrophilic and has two putative bipartite nuclear signaling domains, indicating that TFNR is a soluble nuclear protein. We also identified the first 544 aa of mouse Tfnr (11 exons) which reveals 79% similarity to TFNR. Amino acids 156-470 show significant homology ($1.5e^{-18}$) to the TFC5 protein, a subunit of the yeast transcription factor TFIIIB, suggesting that TFNR may function as a transcription factor. The establishment of TFNR-antibodies is in progress and should draw new light into the cellular location and function of the protein.

Program Nr: 1040 from the 1999 ASHG Annual Meeting

Characterization of a gene encoding an E-rich protein (ERP) expressed in human bone marrow stromal cells.
L.M. King, T. Iwata, C.A. Francomano. NHGRI/NIH, Bethesda, MD.

The identification and characterization of genes expressed in skeletal tissue is one goal of the Skeletal Genome Anatomy Project (SGAP). Bone marrow stromal cells (BMSC) are a population of pluripotent cells capable of differentiating into at least four types of connective tissues: bone, cartilage, hematopoiesis-supporting stroma, and adipocytes. Here we describe the structure, chromosomal location, composition, and expression of a novel gene encoding a glutamic acid (E) rich protein (ERP) isolated from a human BMSC cDNA library. We sequenced the 1.2 kb insert of the clone and extended the 5' and 3' cDNA ends by 5' RACE and by a homology search of 3' ESTs in GenBank. The 1865 bp cDNA sequence contains an 846 bp ORF and based on comparisons with genomic sequences, the gene lacks introns. Four potential poly(A)+ addition sites are present in the 3' UTR of the cDNA. Based on fluorescence *in situ* hybridization (FISH), the gene maps to the cytogenetic location Xq13. We performed Northern analysis to assess mRNA expression across several tissues. The major sites of expression are fetal and adult liver and kidney. Multiple transcripts occur in adult heart. Analysis of the 282 amino acid predicted protein shows that it is largely composed of charged residues: glutamic acid (n=73, 25.9%), lysine (n=51, 18.1%) and aspartic acid (n=29, 10.28%). The N-terminal region contains a consensus bipartite nuclear localization signal (NLS), which is composed of basic residues and has been shown in other proteins to be required for nuclear translocation. To further identify possible functions of ERP, we searched for a mouse homologue of ERP. We found the mouse GARP34 protein, which shows 56.7% similarity to ERP. It is similar in size (307 amino acids), possesses a bipartite NLS and a similar composition of charged residues. GARP34 is expressed in liver and kidney, similar to ERP. GARP34 is also expressed in thymus, small intestine and testis. RNA *in situ* hybridization experiments are in progress to evaluate further the patterns of gene expression in murine development.

Program Nr: 1041 from the 1999 ASHG Annual Meeting

Genomic structure and complete genomic sequences of the Fukuyama-type congenital muscular dystrophy (FCMD) gene, *fukutin*. *K. Kobayashi[1], J. Sasaki[1], E. Kondo-Iida[1], Y. Nakamura[2], T. Toda[1]*. 1) Laboratory of Genome Medicine, Human Genome Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan; 2) Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan.

Fukuyama-type congenital muscular dystrophy (FCMD), one of the most common autosomal recessive disorders in Japan, is characterized by severe muscular dystrophy in combination with cortical dysplasia. We have recently identified the *fukutin* gene responsible for FCMD by positional cloning, and found that the ancestral mutation is the insertion of a 3-kb retrotransposon element. The gene encodes a novel 461 amino-acid protein, fukutin, that is likely to be an extracellular matrix component. In this study, we determined complete genomic sequences that span more than 130 kb around the *fukutin* gene on 9q31, and obtained the complete structure of the gene. The *fukutin* gene extends over 100 kb and is organized into 10 exons ranging in size from 60 to 6,067 bp. The nine introns vary from 1,842 to 16,778 bp, and all splice acceptor and donor sites conform to the AG/GT rule. The flanking 5' region contains a predicted TATA box. More than ten minor transcripts were found that were produced by alternative splicing. We also found two EST clusters constructing two novel genes in the upstream of the *fukutin* gene. These data provide fundamental information for further studies including mutation search, expression regulation, and identification of new genes.

Program Nr: 1042 from the 1999 ASHG Annual Meeting

Genomic organization and chromosomal mapping of the human LALP70 gene. *C. Kosan[1], A. Biederbick[2], H.P. Elsässer[2], J. Kunz[1]*. 1) Institut fuer Humangenetik, Philipps-Universitaet Marburg, Marburg, Germany; 2) Institut fuer Zytobiologie und Zytopathologie,Philipps-Universitaet Marburg, Marburg, Germany.

LALP70 is a human lysosomal apyrase, highly similar to a human uridine diphosphatase (hUDPase), which is located in the Golgi complex. Here we report the analysis of the LALP70 gene structure using a genomic clone which was isolated from a human PAC library by hybridizing with PCR derived probe amplified with LALP70 cDNA specific primers. The LALP70 gene spanns a region of approximately 16 kb and contains 12 exons and 11 introns. All exon/intron boundaries were determined and obeyed the GT-AG paradigm. FISH analysis showed LALP70 to be located on human chromosome 8 in the region 8p21.1-p21.3. To map the gene more precisely the Genbridge G4 radiation hybrid panel (Research Genetics, Huntsville, AL) was used. We determined that the gene is located 0.4 cR distal to WI-961 (LOD > 3.1) which maps in the Genethon map between the genetic markers D8S1734 and D8S1820 (44.9 cM and 54.2 cM from the top of chromosome 8, respectively). Our results suggest that the uridine diphosphatase gene (hUDPase) is a splice variant of LALP70, lacking 24 basepairs. Possible roles of the resulting 8 amino acid stretch for intracellular localisation and enzyme substrate specificity are discussed.

Program Nr: 1043 from the 1999 ASHG Annual Meeting

Identification and Characterization of a brain-specific *APC* homologue, *APCL*(*APC*-like). *K. Koyama[1,4], H. Nakagawa[2], Y. Murata[3], T. Akiyama[3], Y. Nakamura[4].* 1) Department of Human Genome Analysis, Japanese Foundation for Cancer Recearch, Tokyo, Japan; 2) The 2nd Department of Surgery, Osaka University Medical School, Osaka, Japan; 3) Department of Oncogene Research, Institute of Microbial Disease, Osaka University, Osaka, Japan; 4) Laboratory Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

We isolated a novel gene, APCL, that showed significant homology to the adenomatous polyposis coli (APC) tumor suppressor gene. This novel gene, located on chromosome 19p13.3, encodes a protein of 2,303 amino acids that is expressed specifically in the brain. The heptad-repeat domain found in the APC protein is well conserved in APCL (45% of the amino acids are identical); therefore APCL is likely also to form homo- or hetero- dimers. Moreover, since the armadillo domain is also well conserved (76% identical), both proteins may interact with the same or similar molecular entities. The central portion of APCL consists of five copies of a 20-amino-acid motif (FXVEXTPXCFSRXSSLSSLS). Like APC, this domain of APCL was able to bind to b-catenin and deplete the intracellular b-catenin pool. A reporter-gene assay revealed that APCL could also regulate interaction of b-catenin with Tcf, although less actively than APC. These results suggest that the APCL protein may be involved in the Wnt/Wingless signal pathway and the identification of a novel relative of APC may provide new insights into the function of APC. However, APCL C-terminus bears only 13% identity in sequence to the carboxyl region of APC protein. To shed light on the functions of APCL, we have been using a yeast two-hybrid system to search for proteins that associate with its carboxyl region. Among 166 cDNA clones isolated from a human fetal-brain cDNA library as candidates for interaction with APCL, 32 encoded parts of p53-binding protein 2 (53BP2). In vivo studies confirmed that this association regulated the cytoplasmic localization of 53BP2 by concentrating it in the perinuclear region. Since 53BP2 also interacts with p53 and Bcl2 and regulates p53 function, our results suggest that APCL might be involved in the p53/Bcl2-linked pathway of cell-cycle progression and cell death.

Program Nr: 1044 from the 1999 ASHG Annual Meeting

Human transcription factor SLUG: genomic characterization and mutation analysis in patients with neural tube defects. *J. Kunz[1], K. Stegmann[1], J. Boecker[1], C. Kosan[1], A. Ermert[2], M.C. Koch[1]*. 1) Institut fuer Humangenetik, Philipps-Universitaet Marburg, Marburg, Germany; 2) Kinderneurologisches Zentrum des Landes Rheinland-Pfalz, Mainz, Germany.

Vertebrate Slug is a highly conserved member of the snail family encoding zinc finger transcription factors homologous to Drosophila snail and escargot. Slug is expressed in neural crest migrating cells during embryogenesis. Here we report the identification, characterisation and chromosomal fine localisation of the human Slug gene (SLUG). Using fluorescence in situ hybridisation (FISH) we determined that SLUG maps to chromosome 8q11.21-q11.23. The transcript is approximately 2.0 kb in length containing a 807 nt open reading frame organised in three exons encoding a protein of 268 amino acids. Human SLUG protein has five tandemly arrayed zinc fingers, four CCHH fingers and one variant CCHC finger, a structure typical for the vertebrate snail family. Alignment of human SLUG with homologs of other vertebrates (mouse, chicken and Xenopus) demonstrates strong conservation. Based on evidence from model systems SLUG appears to play a role during neural tube closure. Characterisation of the genomic structure including determination of the exon-intron boundaries provided the opportunity to test this hypothesis in humans. We screened the coding sequence of SLUG in 150 patients with neural tube defects (NTD) using single strand conformation analysis (SSCA). We identified a missense mutation (D119E) in one female patient. Thus, our data do not support a major role of SLUG in inducing NTD, even if SLUG may have acted as a predisposing factor in this patient.

Program Nr: 1045 from the 1999 ASHG Annual Meeting

Identification of FLRT1, 2 and 3: A family of novel leucine-rich repeat proteins. *S. Lacy, L.M. Kunkel.* Genetics, Boston Children's Hospital, Boston, MA.

A degenerate oligonucleotide screening assay was used to identify novel proteins which reside in the extracellular matrix, resulting in the isolation of three genes encoding related proteins termed FLRT1, FLRT2 and FLRT3. FLRT1 and FLRT2 are complete sequences, whereas FLRT3 at present is an incomplete clone representing only the 3' end of the complete cDNA. The FLRT1 and 2 open reading frames are each 2 kb in size and code for proteins of approximately 90 kDa. All three proteins are predicted to be transmembrane by hydropathy plot. FLRT1 and FLRT2 each contain 10 leucine rich repeats (LRRs) which constitute almost one half of their sequence; a putative signal sequence is also present at their amino termini. In addition, all three sequences contain fibronectin III and collagen XII-like repeats in the region between the leucine-rich repeats and the transmembrane domain. The LRRs of FLRT1 and FLRT2 are flanked by highly conserved cysteine-rich clusters which are presumed to give rise to intramolecular disulfide bonds and are believed to be present in portions of proteins which reside extracellularly. Potential N-linked glycosylation sites appear twice in the FLRT1 sequence (249N and 305N), 5 times in the FLRT2 sequence (203 N, 299N, 390N, 433N and 521N) and once in the partial FLRT3 clone (106N). The overall structure of the FLRT proteins is similar to several LRR proteins, including human garpin and GP1b-a, Drosophila tartan and gp150. On multi-tissue Northern blots, the 4.4 kb FLRT1 message is expressed in brain and kidney. The 7.5 kb FLRT2 message is highly abundant in pancreas and less prominent in skeletal muscle, brain and heart. The 4.4 kb FLRT3 message is present in kidney, skeletal muscle, lung and brain and lower levels appeared in pancreas, liver, placenta and heart. Overall, the FLRT family of proteins appears redundant. Following FISH and YAC mapping, the chromosomal localization of FLRT1 was determined to be 11q12-13, FLRT2 to 14q24-32 and FLRT3 to 20p11. In summary, the structural composition of the 3 FLRT family members classifies them as novel type I transmembrane proteins and suggests they may be involved in cell adhesion or function as receptors in the context of the extracellular matrix.

Program Nr: 1046 from the 1999 ASHG Annual Meeting

The effect of sequence heterology on recombination between human a**-globin genes.** *J. Lamb, A. Allen, J.B. Clegg.* Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, UK.

Meiotic recombination in the human genome is not randomly distributed and the factors affecting recombination are not well understood. Eukaryotic recombination has been extensively studied in yeast where it has been demonstrated that the introduction of a limited number of sequence heterologies reduces homologous crossovers while increasing aberrant recombinants, including unequal crossover, due to a second recombination event. The advent of sperm typing by PCR has allowed examination of large numbers of human meioses. We have studied the effect of sequence heterology on recombination at the human a-globin complex. The complex contains a duplicated pair of a-globin genes (aa); aberrant recombination due to misalignment of the a-globin genes and unequal crossing-over results in chromosomes with one (-a) or three (aaa) genes as reciprocal events. The a-globin genes are virtually identical at the sequence level but divergence beyond the genes has allowed us to develop a small pool PCR assay to specifically amplify the deletion gene (-a). The recombinant product is visualized by a second round of PCR. Nine polymorphic sites across the a-globin complex can be organized into haplotypes. We have determined the a-globin haplotype for three normal males (2 heterozygotes and 1 homozygote) as an indicator of sequence heterology. The equivalent of $3.8x10^5$ single sperm cells have been analyzed by small pool PCR. These initial experiments gave an estimated recombination rate of $1.4x10^{-3}$ for the homozygote while heterozygotes with 3/9 or 5/9 polymorphisms gave a rate of ~2 $x10^{-3}$. These data suggest that sequence heterology does not increase aberrant recombination at the a-globin locus. In fact, the highest rate was obtained with germ-line DNA from a homozygote. Recombination rates that we have measured in the male germ-line are lower than the frequency of the aaa chromosome in the extant Caucasian population (~1%). Whilst the -a chromosome is under selection in areas of malarial endemicity due to protection against infection it is unclear if selection can account for the aaa gene frequency or if stochastic processes may be acting at the population level.

Program Nr: 1047 from the 1999 ASHG Annual Meeting

Teaching professional ethics to science majors in an undergraduate human genetics course. *A.J. Ahern-Rindell.* Biology Department, University of Portland, Portland, OR.

Professional Ethics is a major area that is missing from many undergraduate science curriculums. Reasons for the lack of coverage are numerous and vary between universities. However, there is an academic ground swelling that recognizes the importance of educating students about the ethical situations that they may encounter in their future careers and professions. The hotly debated question is how professional ethics should be addressed in the classroom and by whom?

Helping students learn the skills for making sound judgements and decisions in their personal and professional lives is a facet of the university's role in educating students. Who is best trained to deal with discipline-specific ethical issues is controversial and therefore, a major impediment in introducing professional ethics into the science curriculum. Anything pertaining to ethical issues is seen as belonging to the realm of the philosophers and is expected to be taught as a humanities course. An other view emphasizes the discipline-specific nature of professional ethics and suggests additions be made to existing science courses on an individual basis.

The latter approach was tried in a science major's Human Genetics course. A part of the course deals with changes to the medical field such as the ability to perform genetic diagnostic testing. For certain diseases, one can determine if a healthy individual may succumb to a fatal, currently untreatable disease later in life. Thus the field of medical genetics and in particular, the genetic counseling profession deals with ethical issues and situations on a daily basis.

In the revised course curriculum, a discussion of the genetic counseling field was augmented by including coverage of ethical situations with case studies, small group and class discussions and a guest genetic counselor. Students responses to the professional ethics material were obtained through surveys and traditional student course evaluations. The students felt their awareness of professional ethics was increased by the course and that their ability to analyze and deal with ethical issues was improved.

Program Nr: 1048 from the 1999 ASHG Annual Meeting

Australian Jewish Women's Experiences with a Breast Cancer Gene Testing Program as Part of the Co-operative Family Registry for Breast Cancer Studies (CFRBCS). **.** *C. Apicella[1,4], L. Andrews[2], K. Tucker[2], A. Bankier[3], M. Friedlander[2], D. Venter[1], J. Hopper[4].* 1) Peter MacCallum Cancer Institute, East Melbourne, VIC, Australia; 2) Prince of Wales Hospital, Randwick, NSW, Australia; 3) Victorian Clinical Genetic Services, Parkville, VIC, Australia; 4) Genetic Epidemiology, University of Melbourne, Carlton, VIC, Australia.

Background and Aims: The Australian Jewish Breast Cancer Study offers testing for the three Jewish founder mutations in BRCA1 and BRCA2. Participants received counselling and results through one of three protocols: 1. Researcher only; 2. Researcher and genetic clinic; 3. Genetic clinic only. Our aim is to evaluate these protocols.

Methods: A questionnaire was mailed to participants excluding those known to be deceased. Genetic knowledge and participant satisfaction questions were modified from those used previously[1,2]. Anonymity of responses was ensured by removing all identifying details prior to access by anyone involved in the study.

Results: Response rate was 75% (156/207). Of respondents, 83% were 'very pleased' to have had the test, and none 'regretted' it. Average scores for genetic knowledge, and for satisfaction with provision of (a) information and (b) emotional support, were high and no different between the protocols. More than 8 in 10 respondents considered information provision as the 'most important' function of genetic counselling, while half considered emotional support as the 'least important'. 90% of respondents raised several other issues through optional qualitative information.

Conclusions: Jewish women viewed information provision as the most important function of counselling, and emotional support provision as the least important. There was no evidence that genetic knowledge gained, or satisfaction with the process, was different between the protocols.

[1]Lerman et al 1996. JAMA 275(24):

[2]Michie et al 1998. J Public Health Med 20(4): 404-8.

Program Nr: 1049 from the 1999 ASHG Annual Meeting

Community education initiatives and the World Wide Web: Experience of the Genetics Education and Counseling Program. *M.G. Banke[1], W.S. Rubinstein[2], E. O'Rourke[1], J.A. Barranger[1]*. 1) Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA; 2) Cancer Genetics Program, Magee-Womens Hospital/UPMC Health Systems University of Pittsburgh Cancer Institute, Pittsburgh, PA.

The Genetics Education and Counseling Program (GECP), a joint effort of the University of Pittsburgh and UPMC Health System, is a community awareness and education initiative on genetics in Pittsburgh and Western Pennsylvania. The GECP extends the community education missions of the Center for the Study and Treatment of Jewish Genetic Diseases and the Cancer Genetics Program. The program is designed to improve awareness of genetics and genetic diseases for the community and for professionals and to provide easy access to clinical and testing services. The GECP offers educational workshops, referral information, brochures, a hotline number (800-640-GENE), and a comprehensive website (http://www.pitt.edu/~edugene). Voluntary workshop questionnaires have shown what the public understands about genetics and what areas require additional resources. Educational workshops in the community present major points regarding why different ethnic populations are at higher (and lower) risks to be carriers for certain genetic disorders, what those disorders are, what genetic counseling and testing involve, and what options people from those ethnic populations have. As a consequence of the educational workshops, new awareness of Tay-Sachs disease carrier frequency has been seen and a new Tay-Sachs disease screening program has been organized in the community. The World Wide Web has proven to serve as a tool for public and professional education. The main webpage of the GECP has received over 6600 visits and averages 10 visits per day. The online Genetics Resource Center (http://www.pitt.edu/~edugene/resource) has received over 8200 visits and averages 22 visits per day. As we reach the start of the new millenium, the World Wide Web and broad-ranging community education initiatives such as the GECP may represent the most advantageous and efficient ways of bringing the explosion of scientific knowledge to the public.

Program Nr: 1050 from the 1999 ASHG Annual Meeting

Counselling in Incontinentia Pigmenti (IP2) families: useful information from X-inactivation studies. *M.L. Bonduelle, W. Lissens, I. Rayen, L. De Raeve, I. Liebaers.* Dutch-speaking Brussels Free University, Brussels, Belgium.

Incontinentia Pigmenti (IP2) is an X-linked dominant neurocutaneous disorder with prenatal male lethality. Affected females display a progressive erythematous and vesicular skin eruption soon after birth evolving through different stages and frequently accompanied by dental and retinal abnormalities. Central nervous system is affected in a variable degree. Expression is highly variable even within families. Penetrance approaches 100%, but in families with minimal symptoms counselling remains sometimes difficult. Selection against mutant alleles in blood leukocytes is a consistent feature in IP2 families (98% in multigenerational IP2 and 85% in new mutations). In families with apparently de novo mutations and minimal symptoms such as dental anomalies in the mother, X inactivation studies can help to make the diagnosis and determine the parental origin of the mutation. In 4 IP2 families where the mother had minimal or no symptoms we determined the parental origin of the new mutation through X inactivation studies for the M27beta locus with restriction enzymes MspI and HpaII. Three new mutations had a maternal and 1 had a paternal origin. Complete skewing in the patients and random X-inactivation in the healthy mother could be used as an additional argument for considering the mother not being a carrier. Unless germline mosaicism would have occurred, these data could be used to reassure the couple and minimalise the recurrence risk for IP2.

Program Nr: 1051 from the 1999 ASHG Annual Meeting

Genetic support groups: Significant others in the conduct of human genetics research. *J.C. Carey[1], J.D. Cody[2], & the ad hoc Committee for Consumer Issues[1,2]*. 1) Dept Peds/Div Medical Genetic, Univ of Utah Medical Ctr, Salt Lake City, UT; 2) Chromosome 18 Registry & Research Society, San Antonio, TX.

The Boards of the ASHG & the Alliance of Genetic Support Groups established the ad hoc Committee for Consumer Issues with the purpose of opening a dialogue between the 2 communities. The first activity of the Committee involved a workshop designed to examine the current conduct of present & future genetics research. Several highly successful approaches to support group/researcher collaboration were identified. A new model of interaction was developed, the essence of which is summarized in the following consensus statement: Research in human genetics is a shared enterprise that involves investigators, participants, & ancillary agencies. The cultures of these groups often differ & these differences create a dynamic tension between shared & divergent interests. When divergent interests interfere with recruitment of participants to research & compromise mutual understanding of the goals of both parties, it is important to identify mechanisms to create or recognize shared goals. Genetic support groups are an important agency in this enterprise. These groups have diverse & differing objectives: support, education, & research. To the extent that successful support groups have created active involvement in research, their activities provide models for future collaborative relationships. The key activities at the level of the support group include education of members about expectations for research, elements of consent, & understanding the nature of research as well as education of investigators in many of the same elements. Support groups can best broker the successful relationships between investigators & participants when the cultural differences are clearly delineated. As the Human Genome Project reaches its objective of a complete sequence & the emphasis in human genetics research evolves from gene finding to identification of disease mechanisms & treatment, the role of support groups will be increasingly important. Active collaboration among investigators, participants, & support groups will be the catalyst for future research productivity in human genetic disorders.

Program Nr: 1052 from the 1999 ASHG Annual Meeting

Drama helps tell our tales. *M.E. Carlin[1], J.R. Redman[2], M.C. Aguilar[3], S.D. McLean[4], G.A. Glenn[5], TEXGENE Ethics and Education Subcommittees, San Antonio, TX[5]*. 1) Genetic & Developmental Ctr. of SW, Fort Worth, TX; 2) NIH, Washington, D.C; 3) CHRISTUS Santa Rosa Health Care, San Antonio, TX; 4) United States Army Corp, San Antonio, TX; 5) TEXGENE Ethics and Education Subcommittees, University of Texas Health Care Center, San Antonio, TX.

For their annual conference, Texas Genetics Network (TEXGENE) highlighted some of the dilemmas posed by the technological advances of genetic presympomatic testing by creating *Genetic Scenes*, a dramatic presentation with narrator and 3 vignettes - a father of teenagers and his wife consider presymptomatic testing for a progressive AD disorder; a single pregnant mom, whose brother died early of an X-linked disease, now carries a male fetus; and a young, chronically ill adult, who is contemplating marriage, learns she has an AR condition. Issues such as testing when there is no known cure, quality of life, longevity, confidentiality and insurability, disclosure to children, prenatal diagnosis costs, loss of income caring for an affected child, alternatives to abortion, revealing inheritability and suggesting testing to a potential mate, having children who could inherit a mutated gene are all brought from the theoretical to immediate personal life experiences by the live drama. The audience reactions and the extended post-presentation discussion, which included the 4 high school thespians, documented the effectiveness of this format in spotlighting the issues, clarifying some of the major questions such situations pose, and enhancing everyone's understanding of the difficult decisions facing those dealing with such dilemmas. *Gene Scenes* has also been presented at a TEXGENE conference for clergy and pastoral caregivers with a similar response. Drama seems to be well suited to assist in educational programs that address personal and family, as well as ethical, legal and social implications of genetic services. TEXGENE will be exploring future productions addressing these issues. (This project was supported by a Maternal and Child Health Grant #MCJ481005.).

Program Nr: 1053 from the 1999 ASHG Annual Meeting

From predictive genetic diagnosis to individually-tailored prevention: Ethical and psychosocial issues related to the identification and management of familial hypercholesterolemia. *L. Caron[1], L. Dallaire[2], G. Bourgeault[3]*. 1) Faculty of Medicine, University of Montreal, Montreal, Quebec, Canada; 2) Service of Medical Genetics Ste-Justine Hospital, Montreal, Quebec, Canada; 3) Faculty of Education, University of Montreal, Montreal, Quebec, Canada.

Objective: In contrast to DNA testing for hereditary breast or colon cancer the identification of individuals at high risk for coronary artery disease (CAD) due to heterozygous familial hypercholesterolemia (FH) has received very little attention even though there are relevant ethical and psychosocial issues to be addressed. **Method:** These issues were explored in a qualitative study using a Grounded Theory approach. Semi-structured interviews were conducted with 10 hereditary dyslipidemia specialists and 15 FH adults attending lipid clinics in the Province of Quebec. Specific themes and FH-CAD explanatory models were elicited to allow for comparative analysis. **Results:** This study shows that knowledge held by most FH individuals closely reflects expert knowledge especially with respect to a) the impact of FH on CAD risk assessment, b) ill-health perception that ensues from attributing a genetic condition c) the acknowledgement of individual responsibility for future health and d) the positive effects of the preventive measures. The treatment rationale still rests on lipid-lowering guidelines which do not account for this specific etiology. **Conclusions:** These results suggest that 1. Informed consent may be viewed as an engagement and nondirective counseling should be revisited; 2. There are negative psychological effects that may result from being unable to achieve "normal" cholesterol levels enlightening the risk of setting unrealistic treatment goals; 3. A major challenge is to define the extent of doctor-patient duties in the context of shared responsibilities for a life-long intervention with uncertain individual outcome and 4. There is a potential for non-caring when dealing with "difficult patients". Overall, the production, communication and use of genetic information is a central issue that should be analyzed more thoroughly from an ethical perspective.

Program Nr: 1054 from the 1999 ASHG Annual Meeting

46,XX males. Genetic Counseling in adults and prepuberal childs: five year follow-up. *G.B. Castyneira[1], S. Gottlieb[2], S. Copelli[2], C. Ruarte[2], C. Perandones[3], M. Mollica[3].* 1) Genes; 2) CEDIE, Hosp de Ninos R. Gutierrez; 3) CENAGEM.

Genetic counseling is not easy in sexual chromosome abnormalities.Patients in which there is not correlation between phenotype and karyotype is even more difficult. We present seven adult patients and three prepuberal childs followed up to postpuberal age. Clinical, hormonal , cytogenetic and molecular aspects were studied. All patients were phenotipic males. In adults hipogonadism was evident in seven patients. They were all azoospermic. In children prepuberal (age 8,9,13) and postpuberal (12,16,17) examinations revealed micropenis in one and gyanecomastia in all the cases. Stature was in the normal range. No other phenotypic abnormalities were found. Their WISC-RIQ were in the bordeline level(70). Hormonal findings showed normal tetosterone levels except in two adults, elevated FSH in four adults, normal LH levels with the exception of one child and three adults. Molecular analysis by PCR showed the presence of SRY gene in all cases. The authors would underline the difficulties of counseling these subjects. When the diagnosis was made in adults the phsycological stress of confirmed infertility and the unavoidably conflict of questioning their sexual identity occurs in all of them. Their process was followed by depression status. In the prepuberal cases the information was based in development, puberty, learning, sexual behavior and fertility. The main conflict was the development and the phenotypic stigmata. During their adolescence they showed poor self image, attentional deficit and emotional impairment. It was remarkable the need of emphathetic support from parents, counselors and pysichologists. Special care was taken to give and explanation that cast no ambiguity on patients social and phenotypic sexual identity. Molecular findings, were very useful in all cases. The aim of these report is to delineate some aspects of genetic counseling in sexual chromosomal abnormalities and highlight the need of a multidisciplinary team to be specially sensitive to the true sexual identity (phenotypic and psychosocial) that is more relevant than chromosomal sex.

Program Nr: 1055 from the 1999 ASHG Annual Meeting

Genetic counseling for azoospermic patients: with focus on reproductive decision in patients with Y chromosome abnormalities. *J.M Chen[1], Y.M Lin[2], P.L Kuo[3], S.J Lin[4].* 1) Department of Behavior Science,; 2) Department of Urology; 3) Department of OBS/GYN; 4) Department of Pediatrics,National Cheng Kung University, Tainan, Taiwan.

Genetic disorders are not infrequent causes of severe oligospermia and azoospermia in male. Treatment with assisted reproduction may result in transmission of the genetic abnormalities in their offspring. Thus genetic counseling is important whether a genetic etiology is known or suspected. Psychosocial counseling and in depth interview with focus on reproductive behavior were conducted in our male infertility clinic. Each azoospermic patient enrolled in the study underwent routine cytogenetic examination, microdeletion screening using Y-linked sequence tagged sites and/or testicular biopsy. Fourteen patients were found to have chromosomal abnormalities among 70 azoospermic patients. Six patients, two cases of 47,XXY and 4 cases of Yq microdeletion, showed strong desire to have children. When asking about any concern on passing genetic defects to offspring, 3 out of 4 patients with Yq deletion did not consider this as a problem and one decided to receive ICSI and two received Chinese herbs treatment. The one who did concern the genetic problem received AID. Two patients with 47XXY chose adoption.

Program Nr: 1056 from the 1999 ASHG Annual Meeting

Providers' knowledge of heritable breast cancer. *G.M. Cohn, D. Cardeiro, L.A. Correia, J. Habecker-Green.*
Ob/Gyn, Baystate Medical Center, Springfield, MA.

Health care providers are vital in determining access to genetic counseling and/or gene testing for individuals at risk for heritable breast cancer(HBC). We surveyed primary care providers and providers of cancer services to address baseline knowledge of and attitudes toward cancer genetics, and methods of risk assessment. Methods: A study group of 307 providers was ascertained through the Baystate Medical Center mailing list of OBGYNs, Oncologists, Internists, General Surgeons, and Psychiatrists. Providers were sent a cover letter, consent, and questionnaire. Upon return, the data was entabulated and evaluated. Results: Overall response rate was poor (15%). Of those responding, each provider reported an average of 52 patients seen at risk for HBC. Average number of patients with whom DNA testing was discussed was 10, and average number of patients referred for genetic services was 6. This indicates at least 70% of patients whom providers believed to be at risk for HBC, did not receive appropriate counseling for these risks. Risk assessment tools such as the Gail, Clause, and Mendelian models were poorly understood, and 65% of providers believed that family members with other cancers were not relevant to a risk assessment. Finally, the genetics of HBC were poorly understood with only 28%; of providers correctly identifying the inheritance of HBC as autosomal dominant and only 52%; believing that the DNA results of an affected relative would be important to their patient's test interpretation. Conclusions: These results suggest that many providers are not ready for the responsibility of providing risk assessment for HBC or informed consent for testing.

Program Nr: 1057 from the 1999 ASHG Annual Meeting

Genethics: Involving non-scientists in the gene technology debate. *F.J. Cunningham[1,2], S. Metcalfe[1,3], M. Aitken[1,2], I. Maccioca[1,2], J. Savulescu[1,3,4], L. Gillam[1,3,4], R. Williamson[1,2].* 1) Murdoch Institute, Melbourne, Australia; 2) CRC for Discovery of Genes for Common Human Diseases, Australia; 3) Dept of Paediatrics, University of Melbourne, Australia; 4) Centre for Study of Health and Society, Melbourne, Australia.

Applications of gene technology cause increasing concern to our community. The public is often exposed to information that is sensationalised or misleading. The Education and Ethics Units at the Murdoch Institute and the CRC have initiated programs that address this by providing a forum for discussion of ethical issues arising from applications of gene technology with 'experts' in the fields of biology, ethics and philosophy. Accurate information is also provided, aiming to improve scientific literacy of the public to facilitate a more informed debate of issues. Our schools program has focussed around a series of 'Ethics Colloquia' involving ethics, philosophy and biology students. Lectures providing background scientific information were followed by discussion of hypothetical scenarios of applications of gene technology, initially in small groups, then a larger forum. Public involvement has been achieved through three activities: 1)A public student debate in the Educational session of the Inaugural Meeting of the Australian Gene Therapy Society to over 200 students and scientists that was preceded by short lectures on gene therapy and its issues. 2) A "Genethics" competition for secondary schools where students expressed their views to a hypothetical scenario on genetic screening for deafness in a written essay. Six finalists presented their arguments to an audience of scientists, students and 'expert judges'. Noted television and radio personalities hosted the finals. 3) An "Ethics of the New Genetics" workshop has enhanced scientific knowledge through a series of lectures and discussion of genetic research scenarios to Institutional Ethics Committee members. Evaluation through analysis of responses to scenarios before and after the program indicated changes in students' attitudes and quality of arguments. Evaluation questionnaires reflect the success of using ethics to promote public interest in genetics.

Program Nr: 1058 from the 1999 ASHG Annual Meeting

Patient perspectives on the process of informed consent for DNA testing. *C.S. Cytrynbaum[1], R. Babul-Hirji[1], S. Kennedy[1], M. Rowell[2], K. Henderson[3], K. Australie[4], H. Druker[1], L. Dupuis[1], N. Quercia[1], C. Shuman[1].* 1) Clinical & Metabolic Genetics, Hosp Sick Children, University of Toronto, Toronto, ON, Canada; 2) Department of Bioethics, Hospital for Sick Children, Toronto, ON, Canada; 3) Division of Medical Genetics, Montreal General Hospital, Montreal QC, Canada; 4) Yale University School of Medicine, New Haven, CT.

The informed consent process (ICP) has long been recognized to be an important component of clinical practice and, more recently, of research as well. Yet limited information is available regarding the impact of ICP on patients and their families. We set out to evaluate patient perceptions of ICP which includes a newly devised DNA testing/banking consent form. In addition to addressing the issues currently recognized to be critical to informed consent, this form allowed individuals to choose between the options of closed consent (sample destroyed after requested testing completed) versus open consent (sample banked for use in ongoing research). Participants completed self-administered questionnaires pre and post ICP. Results are available from 50 completed questionnaires to date. Seventy-six percent of participants chose open consent and 24% chose closed consent. Seventy-two percent indicated an interest in a detailed discussion in the pre-counseling questionnaire and 76% responded that the ICP was helpful in the post-counseling questionnaire, although 83% indicated that they had decided to proceed with testing prior to ICP. Of note, 68% agreed with the statement "consent forms are mostly to provide legal protection for health care professionals". Anxiety levels ascertained by visual analog scale revvealed no significant change in anxiety levels pre and post ICP in half of the respondents. The remaining respondents reported incresed or decreased anxiety levels with approximately even distribution. Preliminary findings indicate a positive attitude towards ICP and provide justification for its implementation in clinical practice. More detailed data analysis, from a larger sample size, will be presented and this information will be utilized to develop guidelines to improve ICP to better meet patient needs.

Program Nr: 1059 from the 1999 ASHG Annual Meeting

Perception of genetic Diseases In a North African Population : Toward the Implementation of Genetic Screening of Hemoglobinopathies. *K. Dellagi, T. Sokraf, A. Ghriss, T. Bellaj, R. Hafsia, M. Bejaoui, A. Hafsia, D.M. Fathallah.* The Hemoglobinopathies Study Group, Institute Pasteur de Tunis-Tunisia. Institut Pasteur Tunis Tunisia.

Over the last decades, considerable progress in controlling communicable diseases has been made in North African countries while awareness of the high prevalence of genetic disorders was increasing. In Tunisia like in most Mediterranean countries, the population has a high frequency of hemoglobin morbid alleles. To identify and evaluate the social and psychological factors that would influence the success of a program to prevent hemoglobinopathies, we conducted a psychosocial survey in a sample of the Tunisian population. Three groups were studied: 82 patients with b-thalassemia major and sickle cell disease, 71 parents, 76 non affected siblings and a control group of 85 healthy individuals. A questionnaire was designed to address the following issues: Knowledge of the disease, prevention and psychological impact.Analysis of the data collected showed an overall poor level of information about the disease. This information was almost lacking in the control group. The majority of the persons surveyed were not informed about prevention however 87%; would be in favor of it, if informed. The remaining 13%; were reluctant because of religious believes. Interestingly the existence of a mandatory premarital testing for Hepatitis B,Syphilis,ABO typing but not screening for sickle cell or thalassemia traits, is approved by 96%; of the persons surveyed. It was striking that audio-visual information means would be best to convey informations on the disease and the prevention. The study also revealed that patients were not given satisfactory psychological support. Interestingly the psychological impact (Self esteem, projection in the future, attitude toward treatment..) varied sharply in patients with b-thalassemia and those with drepanocytosis, the latter being much more negatively affected by the disease.Our study will be helpful in designing effective guidelines for information, testing and counseling programs to control prevalent genetic disorders in other countries of this part of the world.

Program Nr: 1060 from the 1999 ASHG Annual Meeting

GENETIC COUNSELING FOR MITOCHONDRIAL DISORDERS. *M.A. Del Vecchio[1], G.L. Matika[1], C.A. Bay[1,2]*. 1) Division of Medical Genetics, Children's Hosp of Pittsburgh, Pittsburgh, PA; 2) University of Pittsburgh School of Medicine, Pittsburgh, PA.

Mitochondrial disorders are being diagnosed with increased frequency, and present clinicians and genetic counselors with many issues to address. Mitochondrial disorders generally result in deficient energy production or availability. Virtually all organs systems can be affected, but most commonly the central nervous, cardiac, muscular and ophthalmologic systems are involved. Mitochondrial disorders are typically thought of as maternally inherited, but all modes of inheritance have been described and need to be considered during the genetic evaluation. When suspicious of a mitochondrial disorder it is necessary to determine the most likely mode(s) of inheritance, explain difficult genetic concepts and provide psychological support. While these roles are typical for a genetic counselor, mitochondrial disorders present a unique set of challenges. The genetic counselor must obtain a very targeted pedigree with special attention paid to the soft signs in family members, such as migraines, seizures, mental retardation, gastrointestinal complaints, chronic fatigue and weakness. Explanations of difficult concepts such as heteroplasmy, oxidative phosphorylation, nuclear DNA, mitochondrial DNA, and nuclear-mitochondrial gene interactions are unique. Providing psychological support can be a daunting task, even to an experienced team. When one makes a diagnosis of a maternally inherited mitochondrial disease in a proband, one usually also makes the same diagnosis in the mother. In many cases prognosis can vary considerably, thus counseling must by necessity be vague as to prognosis. Frequently there is no reliable prognostic indicator. Like with other genetic disorders the diagnosis is often accompanied by feelings of grief, despair and guilt. While those feelings are not specific to maternal line inheritance, the high recurrence risk in maternal line disorders is specific. Reliable prenatal testing is not available for many mitochondrial disorders, and reproductive options are limited. In summary, mitochondrial disorders present unique and difficult issues for the genetic counselor.

Program Nr: 1061 from the 1999 ASHG Annual Meeting

Consanguinity and birth defects. *B. Dott[1], Y. Alembik[1], M-P. Roth[2], C. Stoll[1].* 1) Serv de Genetique Med, Hopital de Hautepierre, Strasbourg, France; 2) Centre Hospitalo-Universitaire, Strasbourg, France.

The risk for birth defects in the offspring of first-cousin matings has been estimated to increase sharply compared to non consanguineous marriages. As a general decline in the frequency of consanguineous marriages was observed in this century, one wonders whether consanguinity is still a factor in the appearance of birth defects in developed countries. Based on our registry of congenital anomalies we tried to answer to this question. In the population studied in North-Eastern France a consanguineaous mating was known in 1.21% of the cases with congenital anomalies, vs.0.27% in controls, ($p<0.001$). The frequency of the malformations recorded paralleled the degree of consanguinity : out of 89 malformed children, 51 were seen in first-cousins mating (10.3 times more frequent than in offspring of non consanguineaous couples), 17 in second-cousins marriages and 18 in more distant relatives mating. Three were uncle-niece marriage. Excluding known mendelian conditions these numbers were 73, 36, 17 and 17 respectively and the corresponding relative risk were 3.68, 3.01, 3.41 and 4.89 respectively. Therefore there is a negative dose-response effect between level of inbreeding and risk of congenital malformations. Consanguineous mothers were more often pregnant than non consanguineous mothers ($p<0.01$) and they had more stillbirths than non consanguineous mothers. These results show that consanguinity is still a factor of birth defects and they must be taken into account for genetic counseling of inbred marriages, in developed countries.

Program Nr: 1062 from the 1999 ASHG Annual Meeting

What is the status of medical genetics in graduate medical education and continued professional development?

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Purpose: Following graduation from medical school, physicians enter residency training. The curricula are determined by the ACGME (Accreditation Council for Graduate Medical Education), represented by five member organizations: the American Board of Medical Specialties, the American Hospital Association, the AMA, the Association of Medical Colleges, and the Council of Medical Specialty Societies. Following residency, physicians are specialty board eligible. Upon completion of board exams, continuing medical education (CME) materials are available. This research looks at medical genetics in graduate education and professional development. The goal is to assess the opportunities for more effective genetic education on the eve of the completion of the Human Genome Project.

Methods: The ACGME directory was searched for the root word 'gene'. CME modules from 96 medical specialty societies' education conferences were reviewed for genetic medicine content. A survey of the same societies assessed current genetic activities and perceived needs in genetics. The content of review materials targeted to exam board applicants was reviewed.

Results: The ACGME directory reveals sporadic genetics within different residency specialties. The specialty society meeting materials reveals the majority of genetics education is research oriented. Little basic genetics, genetic counseling, or ethical issues raised by genetics is available. Specialty survey results reveal (92% response) 64% have no CME genetic modules, but 67% are interested in developing modules. The specialty board survey results are pending.

Conclusions: Genetics in graduate education is fragmented. It is difficult to be assured of its consistency despite occasional specific references, mainly in pediatric and obstetric/gynecology programs. The latter traditionally have recognized the importance of genetic medicine. There is a great need to fully assess the methods used to teach this 'new science' of medical genetics and particularly its appropriate integration into clinical practice.

Program Nr: 1063 from the 1999 ASHG Annual Meeting

Science for the Next Millenium: High School Cancer Genetics Project. *P.E. Gregory, S. Nolan.* Dept Human Cancer Genetics, Ohio State Univ, Columbus, OH.

The OSU Human Cancer Genetics Program developed a model educational program that provides high school students with both a basic background in cancer genetics and hands-on research experience using PCR. Using PCR kits which are designed specifically for the high school classroom, students isolated DNA samples from their own cheek cells an compared their genetic "fingerprint" to that of DNA from tumor cell lines. The partnership was with four high schools Columbus, Ohio and approximately 200 Advanced Placement biology students participated. This partnership represents an innovative effort to bridge the gap between OSU and the schools. The primary goal of this project was for students to begin to understand that there is a genetic component to all cancers and that some cancers are clearly hereditary. All participating schools were required to do a poster presentation of their research to OSU Comprehensive Cancer Center scientists on campus at the end of the school year. Student evaluations revealed that the students felt well-prepared for the activity (86%) and that the experiments were at the right level (93%). Interestingly, the students were more interested looking at their own DNA than in the DNA from the tumor (71%), this is a something to be considered when planning such a program. The students did well on knowledge questions, with two exceptions: 71% thought PCR could copy the entire genome and 68% incorrectly identified the percentage of cancer that is hereditary (80% overestimated). 80% of the students understood that 100% of cancer cells are genetically abnormal, an extremely important concept for them to grasp. This program was highly successful and will grow to include four more schools during the 1999-2000 school year. Our data strongly suggest that high school students are interested in cancer genetics and research. This is an excellent level to begin public cancer genetics education programs.

Program Nr: 1064 from the 1999 ASHG Annual Meeting

Genetic awareness of consanguinity in a Pakistani Community in the U.K. *H.S. Hasan[1], D. Craufurd[2].* 1) Ctr Medical Ethics, Univ Oslo, Oslo, Norway; 2) Clinical Genetics Dept, St Mary's Hospital, Manchester, UK.

Previous studies have shown children of Pakistani couples in the UK have higher rates of neonatal mortality, congenital malformations, non-specific autosomal recessive diseases and chronic illnesses. High rates of cousin marriages within this community are thought to explain this. This study assessed the attitudes of parents (by semi-structured interviews) to determine the awareness of genetic implications of consanguinity, acceptability of genetic counselling and views regarding second cousin marriages.

Parents of children with a congenital disorder (36 cases) and parents with healthy children (28 controls) were interviewed. The consanguinity rate was found to be 75% in the cases and 46% in the controls ($p<0.005$). Although the majority of the study group had a family tradition of cousin marriages, most were unaware of the congenital malformation risks associated with consanguinity. The majority of the study group thought genetic counselling should be available to all cousins wishing to marry, regardless of their own acceptability of the risk figures. Attitudes towards genetic knowledge and the prospect of community education are discussed as well as a educating young adults in a sensitive non-threatening (both towards community and cultural ties) manner. Views on marriage partners for the study groups' children are analysed as well as the paradoxical views regarding second cousin marriages.

Comments by mothers regarding the psychosocial dimension of their child's genetic disorder are focussed on by the use of specific case mother presentations.

Program Nr: 1065 from the 1999 ASHG Annual Meeting

Critical Predictive Factors in a Woman's Decision to Elect Genetic Testing for Breast and Ovarian Cancer. *G.D. Havens, D. Puñales-Morejon.* Department of Medical Genetics, Beth Israel Medical Center, New York, NY.

DNA testing for mutations in the BRCA1/2 genes is offered to an increasing number of women and families. A woman who knows her mutation status has a more accurate estimation of her risk for breast and ovarian cancer. This knowledge can be useful in devising an individual cancer screening program, altering diet, using medications to decrease the chance of certain cancers, or undergoing prophylactic removal of the breasts and/or ovaries. In addition, if an individual is identified as a mutation carrier, other family members may also be at risk for being mutation carriers and be able to make decisions regarding testing for themselves. However, little is known about the psychological, social and familial characteristics of the women that elect versus decline such testing. Several studies have described women considering mutation analysis, yet few have extended research to those that are actually accepting or declining testing.

Experience shows that not all women who are at risk of carrying a mutation in the BRCA1/2 genes elect DNA analysis. The reasons for declining testing are varied. Some women do not want to live with the knowledge that their risk of breast cancer may be as high as 85% and their risk of ovarian cancer may reach 60%. A woman may feel she is doing everything that she deems acceptable in an attempt to prevent or detect cancer. Others are distrustful of technology and fear harm such as a breach of confidentiality or insurance discrimination.

A group of more than 90 women who received genetic counseling and considered participation in a research protocol that provides free testing for three mutations in BRCA1 and BRCA2 were studied. Data analysis revealed that 65% of the women elected testing after receiving genetic counseling. These women are described according to demographic variables, family history, and primary reasons for electing or declining mutation analysis. Various predictive factors in this sample and recommendations for future research are explored. This information has practical value for genetic counselors as these professionals facilitate decision-making processes for patients.

Program Nr: 1066 from the 1999 ASHG Annual Meeting

Why Patients Don't Show for Their Appointments at a Genetics Clinic. *L. Humphreys[1], A. Hunter[2], A. Zimak[2], M. Cappelli[1]*. 1) Department of Psychology,Children's Hospital of Eastern Ontario Ottawa, Ontario, Canada; 2) Department of Genetics, Children's Hospital of Eastern Ontario, Ottawa, Ontario, Canada.

Cancellations and “no-shows” at outpatient medical clinics represent a significant problem in the delivery of health care services. The purpose of this study was to determine some of the variables associated with non-attendance at the Genetics clinic of the Children’s Hospital of Eastern Ontario (CHEO), in order to find ways to improve the dissemination of genetic risk information and reduce wasted preparation time. A 37-item telephone survey was administered to 62 patients who did not show up for their scheduled appointments and 75 patients who did attend their appointments (or who cancelled with greater than 12 hours notice). The survey was designed to assess a variety of information, including details about the referral source, issues of transportation and child care, beliefs and attitudes regarding genetic health services, and sociodemographic factors. Analyses revealed that patients who attended their appointments perceived more benefits and fewer disadvantages of attending the genetics clinic, and viewed their appointments as being more important than did nonattenders. Attenders also had a higher mean education level and were more likely to be planning on having more children in the future than nonattenders. Practical issues such as having to arrange for childcare and not being paid for time off work to attend clinic appointments were raised significantly more often by nonattenders. These results provide a variety of possible strategies for reducing the no-show rate at this and other genetics clinics. They also suggest ways of improving the quality of care and information provided to patients before, during and following clinic visits.

Program Nr: 1067 from the 1999 ASHG Annual Meeting

APOE genotype and response to galantamine treatment in Alzheimer's disease. *J. Aerssens, P. Raeymaekers, S. Lilienfeld, W. Parys.* Janssen Research Foundation, Beerse, Belgium.

It has been reported that APOE genotype might affect the response to treatment with the acetylcholinesterase inhibitor tacrine in Alzheimer's disease (AD). Our aim was to evaluate whether similar differential effects related to APOE genotype can be observed during treatment of AD patients with galantamine, a cholinesterase inhibitor and nicotinic receptor modulator. APOE genotype was determined in DNA samples from 835 Caucasian AD patients enrolled in two international clinical trials (cohorts of 310 and 525 subjects respectively). These trials were specifically designed to evaluate efficacy of galantamine treatment in AD patients (identified using NINCDS-ADRDA criteria) during periods of 3 and 6 months respectively. In addition to patient demographics, baseline scores for the mini mental state examination (MMSE) and disability assessment for dementia (DAD) were recorded. Scores on the cognitive AD assessment scale (ADAS-cog) were recorded at the start, periodically during and at the end of the clinical trials. APOE genotype and allele frequencies were similar to other AD patient populations. A higher copy number of APOE-e4 alleles was significantly associated with a decreased age of onset of cognitive problems and age of AD diagnosis. No significant associations were found between APOE genotype and baseline scores of MMSE, DAD, or ADAS-cog, nor was APOE genotype associated with the change in cognitive ADAS over 3 to 6 months in the subgroup of placebo treated patients. Overall, a highly significant positive effect of galantamine treatment was observed on the change of ADAS-cog (daily treatment dose: of 24-32 mg). However, no significant differential effect of galantamine treatment was identified after stratification of the AD patients according to APOE genotype or APOE e4 allele copy number. These results were observed in both of the independent trials. In conclusion, our results confirm a strong relationship between APOE and age of onset of AD, but do not support the hypothesis that APOE genotype may be a prognostic indicator of response rate to therapy with galantamine, as has been suggested for other cholinesterase inhibitors in AD patients.

Program Nr: 1068 from the 1999 ASHG Annual Meeting

Molecular evolutionary studies of *HLA-G* null allele suggest non-neutral evolution. *C. Aldrich[1], M.S. McPeek[2], A. Di Rienzo[1], C. Ober[1].* 1) Dept of Human Genetics; 2) Dept of Statistics, Univ Chicago, Chicago, IL.

HLA-G is an unusual class I gene that is primarily expressed in fetal tissues at the maternal-fetal interface, that has membrane and soluble splice forms, and limited and conservative polymorphism at both the amino acid and nucleotide level. A deletion of a single cytosine in exon 3(alpha 2 domain) results in a null phenotype for the full-length protein in homozygotes, and is a striking departure from the otherwise low level and conservative nature of polymorphism in *HLA-G*. Interestingly, this null allele occurs at similar frequencies (0.06-0.08) in distantly-related populations, such as the Spanish and Africans, suggesting that it may have been subjected to evolutionary forces other than random genetic drift. To test this hypothesis we examined variation in genomic regions around *HLA-G* to determine if the pattern of variation around the deletion allele differs from that of a conservative polymorphism in *HLA-G* (T31S), which occurs at a similar frequency in our study population. An African American sample (N=95) was genotyped for the *HLA-G* polymorphisms and for seven flanking STRPs. Age estimates were determined for each polymorphism based on the decay of linkage disequilibrium (LD) (Risch *et al., Nat. Genet.* 1995;9:152) and from allele frequencies assuming neutrality (Kimura and Ohta *Genetics* 1973;75:199). Haplotype frequency estimates were generated by a maximum likelihood algorithm implemented in the HAPLO program (Hawley and Kidd *J. Hered.* 1998;86:409). Based on allele frequencies, the age of the T31S and of the null mutation is approximately 9,500 generations (or about 200,000 years). Consistent with this age estimate, there is no significnat LD between T31S and an STRP at 40kb (D'=0.31,p>0.05). In contrast, significant LD was observed between the null allele and the same flanking STRP (D'=1.0,p=0.001), providing an allele age estimate of only 240 generations, or 5,000 years. These data suggest that these two polymorphisms in the *HLA-G* gene have had different evolutionary histories, and that natural selection may have increased the frequency of the null allele. (Supported by HD21244 and HD27686.).

Program Nr: 1069 from the 1999 ASHG Annual Meeting

Association of pyruvate kinase (ATT)n microsatellite polymorphism with Gaucher disease mutations. *O.M.O. Amaral, E.M. Pinto, M.C. Sa Miranda.* Enzymology Department, Instituto Genetica Medica Jacinto Magalhaes and Instituto Biologia Molecular e Celular, 4150-180 Porto, Portugal.

Gaucher Disease (GD is a lysosomal storage disorder (LSD) where the defective activity of the lysosomal glucocerebrosidase (GBA) leads to subsequent accumulation of glucosylceramide. In Portugal, GD is the most frequent LSD, GBA mutations N370S, G377S and N396T (which has only been found in the Portuguese) together account for about 72% of the alleles among unrelated patients. Previous reports have shown the existence of linkage disequilibrium between pyruvate kinase (PKLR) polymorphisms and GBA mutations (Beutler et al, 1994; Glenn et al, 1994; Rockah et al, 1998; Diaz et al, 1998). Given the relatively high frequency of the aforementioned mutations in the Portuguese patient population, and the fact that they always appeared in the context of the GBA Pv1.1- haplotype, their association with different forms of a polymorphic microsatellite in the PKLR gene was studied. Additionally, the association between this PKLR marker and other less frequent GBA mutations was also investigated. In accordance with the results of other groups, allelic association was verified between mutations in the GBA and PKLR genes. In contrast with findings in the Ashkenazim (Rockah et al, 1998), but in accordance with previous reports (Diaz et al, 1998), mutation N370S was found to be associated not only to 17 repeats but also to 18 repeats. Interestingly, founder effects seemed to exist in the case of two mutations of probable Iberian origin: G377S was always associated to (ATT)17 and N396T was found to be associated to (ATT)14. On the basis of the present study, other rare mutations are also likely to have common origins. The study of PKLR polymorphisms in patients described in the literature, who share uncommon GBA mutations with the Portuguese, could be relevant to investigate the possibility of common ancestors among patients with apparently different ethnic origins.

Program Nr: 1070 from the 1999 ASHG Annual Meeting

Molecular epidemiology of genetic mutations that predispose to thrombophilia in a Greek-Cypriot population. *K. Angelopoulou[1], C. Constantinou[1], A. Chrysanthou[2], A. Nicolaides[1], C. Deltas[1].* 1) The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus; 2) The Cyprus Institute of Reproductive Medicine, Nicosia, Cyprus.

Mutations in several genes have been shown to predispose to an increased risk for thrombophilia. The best studied are the R506Q factor V Leiden mutation, the C677T mutation in the methylenetetrahydrofolate reductase (MTHFR) gene and the G20210A mutation in the prothrombin gene. All three DNA variants have been documented to be significant risk factors for various cardiovascular conditions, including pregnancy complications. We investigated the prevalence of these mutations in the Greek-Cypriot population by screening 90 unrelated individuals. Eleven (12.2%) were heterozygous and one was homozygous (1.1%) for the 506G allele of factor V. In total, 12 subjects (13.3%) carried this mutation, and the mutant allele frequency was 7.2%. Heterozygosity for the MTHFR C677T mutation was detected in 40 subjects (44.4%), whereas 16 of them (17.8%) were homozygous. The mutant allele frequency reached 40%. The G20210A mutation in the prothrombin gene was found in 7 individuals (7.8%). No homozygotes were identified and the mutant allele frequency was 3.9%. Of the 90 subjects 50 (55.6%) had a mutation in either one of the three factors, 11 (12.2%) had a mutation in two of them, and 1 (1.1%) carried a mutation in all three. These data demonstrate that Greek-Cypriots have an increased mutant allele frequency for factor V Leiden compared to the rest of the Europeans (3.2%), and the frequency of the prothrombin mutation is up to 6.5-fold higher compared to the UK population (according to one report). Whether this reflects genetic or environmental factors, or both, is unknown, but these findings are important for alerting physicians in the direction of preventing venous thromboembolic disease and its complications. Assessment of risk factors can also help to decide who requires anticoagulation during surgery or pregnancy. This is the first study for the frequency of mutations in risk factors that predispose to thrombophilia on the island of Cyprus.

Program Nr: 1071 from the 1999 ASHG Annual Meeting

Dissecting inheritance pattern predisposing to vitiligo in Colombian pedigrees. *M. Arcos[1], E. Parodi.[2], E. Bedoya[2], D. Jaramillo[2], G. Ceballos[2], A. Uribe[2], M. Salgar[1], J.J. Builes[1].* 1) Dept Biol, Univ de Antioquia, Medellin, Colombia; 2) Dermatology Service, Internal Medicine Department, Hospital San Vicente de Paul, Medelln, Colombia.

The unified model of segregation was used to elucidate cohort, multifactorial and major gene components predisposing to vitiligo on extended and multigenerational pedigrees from Antioquia, Colombia. Fifteen hypothetical models were contrasted. The hypotheses of cohort effect, recessive major gene, non-polygenic component (against recessivity) in the mixed model and that of non- transmission of major effect were rejected. Multifactorial, major gene (dominant and codominant), no polygenic component (against dominant and codominant major gene in the mixed model) and that of recessive major gene (in the mixed model) hypotheses, could not be rejected. The most parsimonious model is that of a mixed model with strong environmental effects on the recessive genotype. Penetrance and risk corrected by onset age showed that affecteds belong to the codominant and recessive genotype in similar proportions. The affection of the aa unaffected genotype could be explained by the two step Knudson hypothesis.

Program Nr: 1072 from the 1999 ASHG Annual Meeting

Genetic association with human survival and aging at the KLOTHO locus. *D.E. Arking[1], M. Macek Jr.[2], A. Krebsova[2], M. Macek Sr.[2], A. Arking[3], I. McIntosh[1], H.C. Dietz[1].* 1) HHMI and Institute Genet Med, Johns Hopkins Sch Med, Baltimore, MD; 2) Institute Biol and Med Genet, Dept Mol Genet - CF Ctr, Univ Hospital Motol and 2nd Sch Med Charles Univ, Prague, Czech Republic; 3) Johns Hopkins Univ, Baltimore, MD.

Homozygosity for null mutations in the *Klotho* gene leads to a phenotype resembling human aging in mice, including atherosclerosis, osteoporosis, emphysema, and age-related skin changes. To determine whether variation in or around the human KLOTHO locus contributes to fitness and/or longevity, we applied two newly characterized microsatellite polymorphisms flanking the KLOTHO gene in population-based association studies. We compared marker allele frequencies of 429 elderly individuals (>75 years) from a stable Bohemian Czech population that survived high levels of infant and childhood mortality (20%) to 611 contemporary newborns drawn at random from the same population (Macek et al., Hum Genet, 1997). For Marker 1 (12 kb 3' of the last KLOTHO exon), we found an overall difference between the two groups ($p < .03$), with allele 17 more prevalent in newborns ($p < .0002$), suggesting a detrimental influence of this allele on human survival. Marker 2 (66 kb 5' of the first KLOTHO exon) allele frequencies also differ between the groups ($p < .11$), with allele 6 more prevalent in newborns ($p < .05$) and allele 9 more prevalent in the elderly ($p < .05$), suggesting both deleterious and protective roles for different alleles. These differences are not dependent on sex. Additional analysis was performed comparing marker allele frequencies in elderly individuals stratified into "SENIEUR - Apparently Healthy" (140 subjects) and "SENIEUR - Inpatient" (289 subjects) categories of the SENIEUR protocol. Marker 1 allele 13 frequencies were found to be significantly different ($p < .02$), with »0.3% frequency in the "SENIEUR - Apparently Healthy", and »3% in the "SENIEUR - Inpatient" groups. The frequency in newborns was also »3%, suggesting that this allele is a determinant of fitness in the elderly, rather than of early mortality. Ongoing studies will determine if and how klotho protein polymorphisms contribute to these observations.

Program Nr: 1073 from the 1999 ASHG Annual Meeting

Comparison of linkage using simple echocardiographic left ventricular (LV) mass versus factor analysis of LV mass in African Americans: The HyperGEN Study. *D.K. Arnett[1], R.B. Devereux[2], A. Oberman[3], D.W. Kitzman[4], M.A. Province[5], D.C. Rao[5]*. 1) Univ. of MN, Minneapolis, MN; 2) Cornell Univ., New York, NY; 3) Univ. of AL, Birmingham, AL; 4) Wake Forest Univ., Winston-Salem, NC; 5) Univ. of Washington, St. Louis, MO.

An increase in the mass of the LV (ie, LV hypertrophy) is a strong predictor of cardiovascular mortality. Animal studies indicate LV mass may be genetically determined (ie, chromosomal regions are linked to LV mass in rats). No linkage information for LV mass is reported in humans. We evaluated the utility of factor analysis scores compared to simple measures of echocardiographic LV mass for detection of linkage as part of the Hypertension Genetic Epidemiology Network (HyperGEN) study of hypertension genetics. Echocardiograms were collected and centrally read to derive estimates of LV mass in 247 African American hypertensive sibpairs. LV mass was defined by the cubic formula [(posterior wall+intraventricular septal wall+LV internal dimension)^3 - internal dimension^3]. Markers (n=387, CHLC8) were typed by the Mammalian Genotyping Service. Initially, a search for quantitative trait loci for LV mass was carried out using a multipoint model-free variance components method implemented in SEGPATH (Province, 1999), and identified regions on chromosomes 3 and 7 with suggestive linkage to LV mass. Using factor analysis, a factor with echocardiographic LV dimensional and functional measures and height, weight, and waist/hip ratio was created. LV mass was adjusted for the same anthropomorphic variables. Linkage analysis for the LV factor scores and LV mass was done only in regions with prior evidence for linkage to avoid multiple comparisons between the two methods. Results for the factor scores and adjusted LV mass at the two linked markers on Ch7 indicate the factor score provides stronger evidence than adjusted LV mass (LOD=2.49 versus 2.14 at 72.3 cM from the p telomere). However, linkage results were weaker on Ch3 for the factor score compared to adjusted LV mass (LOD=1.80 versus 2.53 at 52.3 cM from the p telomere). We conclude that factor analysis does not provide consistently better linkage results for LV mass phenotypes.

Program Nr: 1074 from the 1999 ASHG Annual Meeting

ATM Mutations in Iranian Patients with Ataxia-Telangiectasia. *M. Babaei, N. Khanlou, M. Mitsui, R. Gatti.* Department of Pathology, University of California at Los Angeles School of Medicine, Los Angeles, California 90095-1732 .

Ataxia-telangiectasia (A-T) is an autosomal recessive disorder with a broad range of clinical manifestations. Epidemiological studies have suggested that A-T heterozygotes are at an elevated risk for breast cancer. A-T occurs worldwide. ATM mutations cover the entire ATM gene, making it difficult to identify heterozygotes in large populations. Here, we report the 11q22-q23 haplotypes of 12 Iranian patients and 3 mutations. Two of these mutations occurred in Iranians of Turkish ethnicity. Although Iranians of Turkish ethnicity are generally considered to be distinct from the people of Turkey, the two mutations and haplotypes were similar to those found previously in A-T patients from Turkey. The third mutation is new. The haplotyping data predict that at least 10 Iranian mutations exist, with the most common mutation accounting for perhaps 40% of affected chromosomes. This would allow genetic screening of large Iranian cohorts for ATM heterozygote identification, similar to studies underway in Costa Rica and Norway.

Program Nr: 1075 from the 1999 ASHG Annual Meeting

Molecular basis of red cell genetic disorders in the Comorian population. *C. Badens[1], F. Martinez di Montemuros[2], M.D. Cappellini[2], D. Lena-Russo[1], J.F. Mattei[1].* 1) genetics, faculty of medicine, marseille, France; 2) Department of Internal Medicine, Ospedale Maggiore, Milano, IRCCS (Italie).

Hemoglobin diseases and G6PD deficiency are red cell genetic disorders which are widespread in various parts of the world. Their geographical distributions correlates well with past and actual malaria distribution because of a selective advantage conferred to heterozygotes against malarial infection. The Comoro archipelagos is located in the Indian ocean and groups 4 islands which have been mainly populated by Bantu Africans coming from Mozambican coasts and by immigrants from the Arabo-Persic gulf, specially from the Shiraz region in Iran. Both countries are areas endemic for hemoglobin diseases and G6PD deficiency but with different underlying molecular defects . For the last fifteen years, Marseilles (France) has been the main destination for Comoro's emigrants. Currently, about 40 000 people from Comoro are living in Marseille. From this population, 11 patients with Sickle Cell Anemia (SCA) and about 80 patients with G6PD deficiency attend the Pediatric Hematology Unit of the Hospital La Timone, Marseilles. In this study, we determine the origin (African or Mediterranean) of the various mutant alleles of the b-globin gene and the G6PD gene, by molecular analysis in a group of comorian patients with hemoglobin disorders and/or G6PD deficiency. The African alleles are predominant being present in all the subjects studied for SCA and G6PD deficiency. Mediterranean alleles have been found for all the b-thalassemia mutations and for two G6PD variants but they are always associated with an African allele in a state of compound heterozygozity. It is noteworthy that the b-thalassemia mutation identified are the most frequent mutation in the Shiraz region of Iran. Our results are consistent well with the mixed Arab-African population of Comoro Islands revealing the presence of unusual heterozygozities composed of East-african and Mediterranean mutations in cases of both hemoglobin diseases and G6PD deficiency.

Program Nr: 1076 from the 1999 ASHG Annual Meeting

Family history of breast/ovarian cancer as an indication of germline BRCA1 mutations. *C.D. Bajdik[1,2], J.M. Raboud[1,3], M.T. Schechter[1,3], B.C. McGillivray[4,5], R.P. Gallagher[1,2].* 1) Department of Health Care and Epidemiology, University of British Columbia; 2) Cancer Control Research, British Columbia Cancer Agency, Vancouver, British Columbia, Canada; 3) Canadian HIV Trials Network, Vancouver, British Columbia; 4) Department of Medical Genetics, University of British Columbia; 5) Women's Health Centre, BC Children's Hospital, Vancouver, British Columbia.

Background: A family history of female breast and ovarian cancer is associated with increased risks for both diseases. In some instances, the family history is due to a germline BRCA1 mutation. Genetic testing requires substantial laboratory resources and expertise, and it would be useful to determine if an individual has a BRCA1 mutation by assessing family history. The accuracy of predictions about BRCA1 will depend on how family history is measured, as well as the age of the proband and the size of the family. Methods: A simple measure of family history was defined by the presence of disease among the proband's close relatives. A second measure was defined by the ratio of observed and expected cases according to population disease rates. Using computer simulations, we estimated the sensitivity and specificity of each measure as a test of BRCA1 mutations. Results: For both measures, the sensitivity of family history depended on the proband's family size, and to a lesser extent on the proband's age. The specificity of the measures also varied with the proband's age and family size, but less so than sensitivity and not in any systematic manner. Tests based on the different measures of family history had similar sensitivity and specificity for probands of the same age and having the same family size. Conclusions: The similarity of results for the two measures is noteworthy because the simpler one's calculation is more practical for health care workers. Our results show that, even in large families and populations where roughly 1% carry a germline BRCA1 mutation, the chance that someone with a family history has a germline BRCA1 mutation is less than one in nine. Many carriers in the population will have no family history of breast/ovarian cancer.

Program Nr: 1077 from the 1999 ASHG Annual Meeting

Genetic evidence on the origins of Indian castes. *M. Bamshad[1], T. Kivisild[2], W.S. Watkins[1], M.E. Dixon[1], B.B. Rao[3], J.M. Naidu[3], B.V.R. Prasad[3], A. Rasanayagam[4], S.S. Papiha[5], R. Villems[2], L.B. Jorde[1]*. 1) Eccles Inst Human Genetics, Univ Utah, Salt Lake City, UT; 2) Inst of Mol and Cell Bio, Tartu Univ and Estonian Biocentre, Tartu, Estonia; 3) Dept of Anthro, Andhra Univ, Andhra Pradesh, India; 4) Lab of Mol Systematics and Evol, Univ of Arizona, Tucson, AZ; 5) Dept of Human Genetics, Univ of Newcastle-upon-Tyne, UK.

The origins and affinities of the 1 billion people living on the Indian sub-continent have long been contested. Shared Indo-European languages (i.e., Hindi and most European languages) suggested to linguists that contemporary Indians are descendants of Caucasians who migrated from Western Eurasia (Europe, the Near East, Anatolia, and the Caucasus) 3,000 to 4,000 years ago. However, archaeological evidence of the diffusion of material culture from Western Eurasia into India has been limited. To reconcile the origin of Indian castes, we analyzed mtDNA and Y chromosome markers to estimate the genetic affinities of castes of varied status to world-wide populations. For maternally inherited mtDNA, the majority of Indian mtDNA lineages belong to an Indian-specific haplogroup, all castes show highest similarity with East Asians, and the level of European admixture with Indians is proportionate to caste rank. This is consistent with either the hypothesis that proportionately more West Eurasians became members of the upper castes at the inception of the caste hierarchy or that social stratification preceded the West Eurasian incursion and that West Eurasians tended to insert themselves into higher-ranking positions. In contrast to the mtDNA distances, the Y chromosome data do not demonstrate a closer affinity to East Asians for each caste group. Upper castes are more similar to Europeans than to East Asians. This is consistent with sex-specific differences in levels of admixture, with males with European affinities contributing proportionately more than females to upper castes. We conclude that Indians are largely of proto-Asian origin, and admixture with West Eurasians was recent and limited in scope. Nevertheless this admixture resulted in rank-related and sex-specific differences in the genetic affinities of castes to East Asians and Europeans.

Program Nr: 1078 from the 1999 ASHG Annual Meeting

Examination of phylogenetic relationships among Siberian and Native American populations using pooled DNA samples. *P. Banerjee[1], J. McComb[2], A. Pfeffer[2], M.H. Crawford[2], J.A. Knowles[3,4]*. 1) Genetics and Development, Columbia University, New York, NY; 2) Department of Anthropology, University of Kansas, Lawrence, KS; 3) Dept. of Psychiatry,; 4) Columbia Genome Center, Columbia University, New York, NY.

A number of studies have attempted to elucidate the genetic origins of the Native Americans. Since the early 1990s, samples from the native populations of Siberia have been examined with polymorphic DNA markers (mtDNA, STRs, VNTRs) and yielded a conflicting picture of the peopling of the New World. One limitation of these studies was the small number of markers examined in each. In an attempt to construct a more accurate phylogenetic relationship of this migration we plan to screen the entire genome of multiple populations with 377 STRs spaced approximately 10 cM apart. To reduce the amount of genotyping required, we pooled equimolar amounts of DNA from each sample from three Siberian (Altai, Buryat and Chuvash), one Native American, one South African, and one Caucasian population and determined the allelic frequencies. In our preliminary analysis of 20 STRs spread across the genome, we have found that the frequency of each allele is accurately estimated by the proportion of height in each peak (r=0.9811). Furthermore, we have corrected for error in measurement using DNA pooling as compared to typing individual samples by linear regression. Using this model of prediction, we obtained 95% confidence limits for the proportions (heights) and compared populations. Populations were considered to be significantly different if at least one of the proportions was different and multiple such differences were observed. We plan to screen the rest of the genome and compare these populations and develop a weighted measure of genetic distance. This distance shall enable us to connect the various populations and establish phylogenetic relationships among them.

Program Nr: 1079 from the 1999 ASHG Annual Meeting

Multiplex heteroduplex generator strategy to genotype homocysteine related polymorphisms. *S. Barbaux, L.A.J. Kluijtmans, A.S. Whitehead.* Pharmacology, University of Pennsylvania, Philadelphia, PA.

A modest elevation of circulating levels of the amino acid homocysteine has been identified as a risk factor for some human pathologic conditions, including cardiovascular diseases and neural tubes defects. We have developed a rapid and simple method for genotyping the 4 most common polymorphisms in genes related to homocysteine/folate metabolism. These polymorphisms consist of 2 missense mutations in the methylenetetrahydrofolate reductase (MTHFR) gene (C677T and A1298C) and 1 in the methionine synthase (MS) gene (A2756G), and an insertion/deletion polymorphism of 68 bp in the cystathionine beta synthase (CBS) gene. The method is based on a multiplexed heteroduplex strategy: for each polymorphism, a fragment identical to the target region, except for a few base pairs deletion close to the mutated nucleotide, is added to genomic DNA prior to PCR amplification to produce heteroduplexes, that have different mobilities depending on the presence or absence of the polymorphic change. Synthetic heteroduplex generators have been generated for each of the above missense polymorphisms and cloned together into a single construct. Primers specific for each polymorphism were designed and reaction conditions optimized to produce heteroduplexes and homoduplexes of diagnostic sizes, that could be resolved on a 12% non denaturing polyacrylamide gel. Thus the above 4 genotypes can be determined by electrophoretic analysis of the products of a single PCR reaction. This method will facilitate further research on the role of genetic variants of genes involved in homocysteine/folate metabolism in various conditions and enable rapid genotyping of at-risk individuals to be undertaken: i) rapidly and at low cost and ii) without the failure and partialing problems inherent in the restriction enzyme based genotyping currently used to define MTHFR and MS variants.

Program Nr: 1080 from the 1999 ASHG Annual Meeting

The impact of racial admixture on traditional linkage analysis. *J.S. Barnholtz[1,2], M. de Andrade[1], R. Chakraborty[2].* 1) MD Anderson Cancer Center, Dept. of Epidemiology, Houston, TX; 2) UT School of Public Health, Houston, TX.

Racially admixed families are routinely excluded from traditional (LOD score) linkage analysis or analyzed as racially homogeneous using the proband's race. Through simulation, we investigated the effect of admixture on the LOD score of two racial groups under various conditions.

Four-generation racially homogeneous and admixed families were simulated with 27 markers and two linked, bi-allelic disease loci. One locus was linked to a marker with correct allele frequencies for both groups and the other was linked to a marker with correct information for only one group. Two different types of admixture were tested: admixture within a family unit and a mixture of homogeneous families within a data set. The mixing was done at the founder level in three different proportions: 30/70, 50/50 and 70/30.

We observed that the LOD scores under both models of admixture were closest to the homogeneous family scores of the racial group having the highest mixing proportion. In the 50/50 case, the LOD scores were in-between the homogeneous scores. Random sampling of families or ascertainment of families with disease affection status did not affect this observation, nor did the mode of inheritance (dominant/recessive) or disease/marker allele frequencies.

LOD scores in admixed family data were further affected due to departures from Hardy-Weinberg expectations of genotypic frequencies and presence of linkage disequilibrium in admixed populations. Subroutines performing these tasks with relaxed assumptions are being implemented in current LOD score analysis programs. (Research supported by NCI-R25CA57730-07 and NIH-GM41399.).

Program Nr: 1081 from the 1999 ASHG Annual Meeting

Large human panels for the study of genomic variation. *P.K. Bender, L.H. Toji, C.M. Beiswanger, J.C. Leonard, J.C. Beck, R.T. Johnson.* Coriell Cell Repositories, Coriell Inst. for Medical Research, Camden, NJ.

The Coriell Cell Repositories have compiled panels of cell lines and DNA samples from 90 donors representing 9 different ethnic origins or geographic regions. These panels of 10 individuals each have stimulated considerable research interest and requests for additional and larger panels of Caucasians and African Americans. Panels of 50 and 100 samples from African Americans have been assembled, and similar size panels of Caucasians will be available shortly. The African American samples were collected principally from the Northeast of the U.S. The samples are from unrelated individuals and most of the samples are from clinically unaffected donors. Approximately, 80% of the samples are female. Based on the geographic data, it is estimated that the European genetic contribution to these African American panels may range between 14-20% (Parra, E.J., *et al.*, *Am. J. Human Genet.*, **63**, 1839-1851, 1998). These data assure that these panels will provide a good control cohort for many linkage studies.

As part of the Repositories' routine quality assurance, each sample has been genotyped with six different microsatellites. This procedure assures sample identity from receipt of sample to shipping. As an introduction to the variation present in these panels, the allele frequencies for these six microsatellites have been determined and are available as histograms. These data allow comparison of the occurrence of low frequency alleles in the different size panels, and provide a basis for comparing representation between the different size panels. The availability of these panels provides a new and extended resource for the identification of polymorphisms that contribute to genetic variation, disease linkage, and anthropological studies. »»

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Program Nr: 1082 from the 1999 ASHG Annual Meeting

Modelling Susceptibility to Bowel Cancer using Segregation Analysis. *D.T. Bishop[1], D.J.B. St John[2], G.P. Crockford[1], E.A. Debney[3], F.T. McDermott[3], E.S.R. Hughes[4].* 1) Gen Epid Lab,St James Univ Hos, Imperial Cancer Research Fund, Leeds, England; 2) Dept of Gastroenterology, The Royal Melbourne Hospital, Melbourne, Australia; 3) Monash University, Dept of Surgery, Alfred Hospital, Melbourne, Australia; 4) Deceased.

Segregation analyses of susceptibility to bowel cancer have uniformly identified a rare, dominantly inherited susceptibility with lifetime penetrance 0.5 or higher. However, it is now known that germline mutations in mismatch repair genes (notably hMSH2 and hMLH1) are associated with susceptibility in families with the syndrome, Hereditary Non-Polyposis Colorectal Cancer (HNPCC). Many of the families with multiple cases of colorectal cancer and especially those with early onset can be attributed to mismatch repair gene mutations while for others this is apparently not the case. We are interested in (i) estimating the contribution of mismatch repair genes to colorectal cancer incidence in the general population and (ii) evaluating the evidence for other genetic factors being involved in bowel cancer susceptibility in families without mismatch repair mutations. To investigate these issues, we obtained detailed and verified family histories from 525 bowel cancer patients of the late Sir Edward Hughes in Melbourne. Four of these families are known to have mismatch repair mutations. Segregation analysis of these remaining families again suggests a rare, high penetrance gene with lifetime penetrance 0.45. Reasoning that many other families may well have mismatch repair mutations but have not or will not be tested, we obtained from other studies (including our own), the contribution of MMR mutations to colorectal cancer incidence by extent of family history. Incorporating this information into the segregation analysis still suggests a dominantly inherited susceptibility but with decreased gene frequency suggesting that there are other genes involved in susceptibility to colorectal cancer.

Program Nr: 1083 from the 1999 ASHG Annual Meeting

Comparing odds ratios from family-based and case-control association studies: application to Alzheimer's disease. *D. Blacker[1,2], L.M. O'Brien[3], R.E. Tanzi[4], N.M. Laird[3].* 1) Dept Psychiatry, Mass General Hosp, Boston, MA; 2) Dept Epidemiology, Harvard Sch Pub Health, Boston, MA; 3) Dept Biostatistics, Harvard Sch Pub Health, Boston, MA; 4) Dept Neurology, Mass General Hosp, Boston, MA.

A consideration of effect sizes as well as p-values can help reduce controversy surrounding association findings in complex diseases, as they allow comparison of an effect's magnitude and direction and are not dependent on sample size. For case-control studies, an odds ratio (OR) can be estimated directly or by using logistic regression to control for known confounders. For family-based studies, where unaffected relatives (e.g., sibs) serve as controls to prevent confounding by admixture, *Mantel-Haenzel* ORs and *conditional* logistic regression, both stratified on family, must be used; *crude* ORs *not* stratified on family are biased downward. Case-control and family-based ORs can differ due to population admixture, ascertainment factors, and other uncontrolled confounders. In the absence of these factors, if--as is likely in complex diseases--multiple genes and other familial factors jointly contribute to risk, family-based ORs will generally be higher than case-control ORs. These points are illustrated below for two Alzheimer's disease (AD) risk factors: the established apolipoprotein E-4 (APOE-4) and the recently reported alpha-2-macroglobulin (A2M-2). We report both conditional and crude ORs on 120 sibships discordant for AD (n=467; p-values are from discordant sibpairs only) and crude ORs on the 120 proband cases vs. 129 cognitively intact elderly controls. Future work will compare multiple samples and consider p-values and confidence intervals for ORs based on all available sibs.

OR (p)	Family, conditional	Family, crude (wrong)	Case-control
APOE-4+	2.5 (.002)	1.9 (.008)	9.5 (.0001)
A2M-2+	2.3 (.03)	1.5 (.08)	1.2 (.43)

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Program Nr: 1084 from the 1999 ASHG Annual Meeting

Analysis of joint mismatch distributions of sequences from mitochondrial hypervariable regions 1 and 2, in a sample of individuals from major world populations. *A. Bobrowski[1], M. Kimmel[2], D.N. Stivers[1], R. Chakraborty[1]*. 1) Human Genetics Center, University of Texas at Houston, Houston, TX; 2) Dept Statistics, Rice Univ, Houston, TX.

We developed a mathematical model, which makes possible to predict joint distributions of numbers of mismatches in two or more regions of the genome, based on the infinite sites models, under mutation-drift equilibrium or various patterns of population growth. One of the predictions is different correlation between numbers of mismatches in the two regions, depending on the pattern of the past population growth (constant, slowly growing, or rapidly growing). In this way, the method provides additional insight into the demographic history of the populations. Also, we developed expectations and variances of sample statistics under different growth scenarios. As an application we used a sample of mitochondrial sequences from hypervariable regions 1 and 2, representing all major world populations (Europeans, Asians and Africans). The patterns of joint distributions of numbers of mismatches markedly differ from one population to another. African mtDNA data are most consistent with rapid expansion, while Asian data seem to indicate early occurrence of population mixture. (Research supported by NIH grants GM 41399, GM 45861, and by the Keck's Center for Computational Biology at Rice University).

Program Nr: 1085 from the 1999 ASHG Annual Meeting

Frequency of *GJB2* (connexin 26) mutations in African American and Caucasian populations in North Carolina.
J.K. Booker, Z. Zhou, L.M. Silverman, E.M. Rohlfs. Dept. of Pathology & Lab. Med., Univ. of North Carolina, Chapel Hill, NC.

More than 80% of individuals with non-syndromic autosomal recessive deafness are either homozygous or compound heterozygous for mutations in the *GJB2* gene that encodes the connexin 26 protein. The most frequently observed mutation is 35delG. Carrier frequencies for 35delG have been shown to vary from 0.73% in the Ashkenazi Jewish population (where 167delT is seen at a frequency of 4%) to 3.2% in individuals from Italy and Spain. In preparation for offering clinical mutation analysis for autosomal recessive deafness, we determined the carrier frequency of the 35delG mutation in North Carolinians, primarily comprised of Caucasians and African Americans. DNA samples collected for unrelated genetic analysis were tested for the 35delG mutation by an allele specific PCR assay. The 35delG mutation was found in 2/500 African Americans (0.4%) and 10/500 Caucasians (2.0%). The 35delG carrier frequency is significantly lower in African Americans than Caucasians ($p=0.02$) and may be the result of admixture. The 35delG mutation is thought to have occurred several times based on haplotype analysis; it is not known whether it occurred in Africa. The only study of the carrier frequency in the United States tested 173 African Americans without finding the 35delG mutation. In addition to the 35delG analysis, we developed a PCR mediated site directed mutagenesis assay to screen for the *GJB2* R143W mutation identified in a village in eastern Ghana with an extremely high frequency of non-syndromic recessive deafness. To date we have tested 200 African Americans for the R143W mutation without detecting it. This study suggests that the 35delG mutation will account for a significant proportion of non-syndromic recessive deafness in the North Carolina population. Complete sequence analysis will be necessary to identify additional *GJB2* mutations, both in Caucasians and African Americans with non-syndromic recessive deafness, with the potential to identify recurrent mutations in the African American population.

Program Nr: 1086 from the 1999 ASHG Annual Meeting

Genetic analysis of schizophrenia in isolated Daghestanian kindreds. *K. Bulayeva[1], K. Roeder[2], S.A. Bacanu[3], P. Bennett[4], B. Devlin[3], B. Byerley[1]*. 1) Department of Psychiatry, Univ. of California , Irvine, CA; 2) Department of Statistics, Carnegie Mellon University, Pittsburgh, PA; 3) Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA; 4) Department of Psychiatry, University of Utah, Salt Lake City, Utah.

Despite differing cultures and environments, the worldwide prevalence and presentation of schizophrenia are remarkably homogeneous. Our goal is to identify genes that underlie susceptibility to schizophrenia in the ethnically diverse population of Daghestan, a Russian republic composed of 26 Caucasoid ethnic groups. Most of the ethnic groups reside in remote highland villages that can be classified as 'primary isolates'. Prolonged reproductive isolation and severe environmental conditions in the highland villages have created genetically diverse isolates in this region (Bulayeva,1991, Genetic Basis of Human Psychophysiology. Moscow:Nauka, 218 p). Due to the substantial endogamy and inbreeding, each isolate is essentially an extended family or kindred. Notably some kindreds have numerous affected individuals, yet others are disease free. These kindreds should prove a valuable resource for genetic analysis of schizophrenia. As part of an ongoing study, 12 large kindreds have been partially ascertained. Of the 292 subjects sampled thus far, 87 are chronic schizophrenic cases primarily from five ethnic groups, Avars, Dargins, Koumiks, Tindalls and Laks. A 10 cM genome screen (CHLC 9.0 markers) using these subjects has recently been completed by the Mammalian Genotyping Service. Due to sparse sampling and inbreeding, linkage analysis on these kindreds is quite challenging. As one approach, we are using descent graph methods developed by Sobel and Lange (1996; Am J Hum Genet 58:1323-1337) and implemented in Simwalk2. Analysis of these large, complex pedigrees has proven tractable, albeit computer-intensive. We present results for whole-genome marker-sharing statistics.

Program Nr: 1087 from the 1999 ASHG Annual Meeting

Epidemiologic phenotype in Irish families with neural tube defects. *J. Byrne*[1,2]. 1) Center VI, Children's Natl. Med. Center, Washington, DC; 2) Boyne Research Institute, Drogheda, Ireland.

This abstract introduces the term "epidemiologic phenotype" in the context of studies of the etiology of neural tube defects (NTDs). Both the epidemiologic phenotype and the clinical phenotype of NTDs are unusually complex and probably heterogeneous in origin. In contrast to the clinical phenotype (the proband's diagnosis), the epidemiologic phenotype can be thought of as those characteristics that affect other family members, or that influence NTD rates overall, i.e., area differences and incidence declines. Both the clinical and the epidemiologic phenotype may be considered as representing the sum of genetic and environmental factors associated with NTDs. Among the features of the epidemiologic phenotype of NTDs previously reported are excess risk for a recurrence in sibships, excess miscarriages in sibships, and preferential maternal inheritance. Some of these characteristics have been related to the clinical phenotype, i.e., high or low spina bifida. This study of Irish families extends our knowledge about the features of the NTD epidemiologic phenotype. With historically high rates of NTDs and relative homogeneity of the population Ireland presents a unique opportunity to study the origins of NTDs. Interviews with 77 Irish NTD families revealed a large number of factors occurring to excess ($p<.05$), including preferential maternal transmission. These are: large family size and excess miscarriages in NTD sibships, excess risk for NTDs and other malformations in sibs, excess birth defects on the maternal side and, for the first time, subfertility and infertility among maternal relatives. NTDs are etiologically complex resulting from the combined action of multiple factors, both genetic and environmental, acting together. Further studies that continue to expand the epidemiologic phenotype and relate it to the clinical phenotype and ultimately to the genotype will enhance our understanding of the contributions of gene and environment to the etiology of NTDs.

Program Nr: 1088 from the 1999 ASHG Annual Meeting

The relationship between IL-1 genotype and clinical response to recombinant IL-1 receptor antagonist therapy in rheumatoid arthritis. *N.J. Camp[1,3], A. Cox[1], F.S. diGiovine[1], D. McCabe[2], W. Rich[2], G.W. Duff[1]*. 1) Molecular and Genetic Medicine, University of Sheffield, UK; 2) Amgen Inc, CA; 3) Current affiliation: Genetic Research, IHC, UT.

Rheumatoid arthritis (RA) is a multifactorial inflammatory disease with a genetic component. Interleukin-1 (IL-1) plays a central role in the pathogenesis of RA, and agents which antagonise its activity can be of therapeutic benefit. One such molecule, the IL-1 receptor antagonist (IL1ra), blocks IL-1 activity by binding to the IL-1 receptor. The genes for IL1alpha, IL1beta and IL1ra are clustered within 430kb on chromosome 2. Using markers in the IL-1 region, we investigated the effect of IL-1 genotype on clinical response of patients with RA to IL1ra treatment, using data from a recent clinical trial of recombinant IL1ra.

A positive response was considered to be a reduction of at least 50% in the number of swollen joints presented by the patient by week 24. The treatment dose considered was 150mg, and 91 patients were included in these analyses. The response rate to treatment, independent of genotype, was 48.4% (44/91). A significant association was found between carriage of the rarer allele at IL-1A(+4845) and response to treatment (p=0.0009, OR=4.85(1.85,12.70)). The response rate for individuals carrying the rare allele at IL-1A(+4845) was 63.4%, compared with 26.3% for those who did not. Associations were also seen between IL-1A(-889) and IL-1B(+3954) and response to treatment, but these were more consistent with linkage disequilibrium with IL-1A(+4845) rather than independent associations or epistatic effects. Logistic regression also confirmed the role of genotype at IL-1A(+4845) in response to treatment. Carriage of the rare allele at IL-1A(+4845) was chosen for inclusion in the model (p=0.0008) with a predicted OR of 5.08(1.97,13.06).

In conclusion, we have found an association between IL-1A genotype and response to IL1ra treatment of RA. This could be used to predict a subset of RA patients who are more likely to respond to therapy with IL1ra (63.4%) than those who do not carry this allele (26.3%).

Program Nr: 1089 from the 1999 ASHG Annual Meeting

The HGDP-CEPH Human Genome Diversity Panel. *H.M. Cann[1], C. De Toma[1], A. Marcadet-Troton[1], G. Thomas[1], J. Dausset[1], L.L. Cavalli-Sforza[2].* 1) Foundation Jean Dausset-CEPH, Paris, France; 2) Department of Genetics, Stanford University School of Medicine, Stanford, CA.

The Human Genome Diversity Project (HGDP) has been organized for the promotion and coordination of studies involving the typing of large samples of individuals from populations throughout the world with hundreds of genetic DNA markers. Such studies promise an improved understanding of human evolution, and are also relevant for mapping genes implicated in complex disorders. The objectives of the initial HGDP project, undertaken in collaboration with CEPH, are to 1) assemble a collection of existing lymphoblastoid cell lines (LCLs) from 1000 individuals from 40-50 populations and 2) develop a set of genetic markers to be used for characterization of the genetic structure of various populations. Each HGDP participant will type the DNA from the LCL panel with local markers, and a common set of markers will also be used for typing by a core group of laboratories. The results of DNA typing will be deposited in a database initially available to participants and later to the public.

Currently the panel contains 367 mycoplasma-negative LCLs stored at -180° C at CEPH. Each LCL is tested locally (optionally) for mycoplasma and on reception at CEPH with a PCR-based procedure. Sixty three mycoplasma+ LCLs have been found and discarded. Geographic origin of populations represented in the current panel are Africa (2 populations), the Middle East (2), Pakistan (8), India, Laos, Melanesia, New Guinea and France. To date DNA has been prepared at CEPH from 318 LCLs, the amounts ranging from 0.7 to 3.5 mg. per cell line (less than 1 mg. for 7 lines). CEPH will send to participants a minimum of 1 mg. of DNA from each of the 1000 LCLs in 96 well format. Identifying information for each LCL will be limited to the sex and geographic and population origin of the sampled individual.

A list of LCL contributors will be presented.

Program Nr: 1090 from the 1999 ASHG Annual Meeting

Comorbidity between psychotic disorders in the Maudsley twin psychosis series. *A.G. Cardno[1,2], F.V. Rijsdijk[3], P.C. Sham[3], R.M. Murray[3], P. McGuffin[3]*. 1) Psychological Medicine, UWCM, Cardiff, UK; 2) Medical Genetics, UWCM, Cardiff, UK; 3) Institute of Psychiatry, London, UK.

Background: Operationally-defined schizophrenia, schizoaffective disorder and mania show evidence of substantial heritability. However, whether these disorders share genetic risk factors in common is controversial. This issue can be investigated by applying biometrical model fitting to data from twins. Methods: One hundred and sixty six probandwise twin pairs (77 MZ and 89 same-sex DZ), where the proband met criteria for RDC lifetime-ever schizophrenia (Sz), schizoaffective disorder (Sa), or mania (Ma) were ascertained from the Maudsley Twin Register in London. Correlations in liability were calculated for each disorder in MZ and DZ pairs, and across the three pairings of Sz-Ma, Sz-Sa, and Sa-Ma, both within twins and within pairs. For each pairing, a correlated liability model was fitted. For all three disorders considered together, independent pathway and common pathway models were fitted. Results: Under the correlated liability model all three pairings of disorders showed significant genetic correlations. The analyses of the three disorders together suggested common and diagnosis-specific genetic contributions to variance in liability to both schizophrenia and mania, but negligible diagnosis-specific genetic effects for schizoaffective disorder. Conclusions: These results are consistent with a degree of overlap in the genes contributing to RDC schizophrenia, schizoaffective disorder and mania, when these disorders are defined on a non-hierarchical lifetime-ever basis.

Program Nr: 1091 from the 1999 ASHG Annual Meeting

Interactions between genetic and reproductive factors in breast cancer risk in African-American women. *V. Chaudru[1], A. Laing[2], G.M. Dunston[2,3], L. Adams-Campbell[2], R. Williams[2], J. Lynch[4], D. LaSalle Leffall[2], R. DeWitty[2], B.L. Gause[2], G.E. Bonney[2,3], F. Demenais[1,2].* 1) INSERM U.358, Paris, France; 2) Howard University Cancer Center, Washington, DC; 3) The National Human Genome Center at Howard University, Washington, DC; 4) Washington Hospital Center, Washington, DC.

Although considerable progress has been made in the identification of breast cancer (BC) genes, BC remains a complex disease involving a major genetic component and other risk factors, mainly related to the reproductive life. We have investigated interactions between genetic and reproductive factors in BC risk in 245 African-American families, ascertained through 245 BC probands followed at Howard University Hospital and Washington Hospital Center (Washington, DC). Information on BC history and reproductive factors (age at menarche, number of children, number of spontaneous and induced abortions, menopausal status) was recorded for the probands and female first-degree relatives. Segregation analysis of BC was conducted by use of the class D regressive model taking into account a variable age of onset of disease, as implemented in the REGRESS program. Our results show significant evidence for the segregation of a rare dominant gene ($p<0.0001$) and additional sister-sister dependence ($p=0.0001$). Age at menarche is found to interact significantly with this gene, and an interaction with spontaneous abortions is suggested. A late age at menarche increases BC risk in susceptible women but has a protective effect in non-susceptible women. A history of spontaneous abortions has a protective effect in susceptible women whereas it increases BC risk in non-susceptible women. These findings agree partially with those previously reported in a French study (Andrieu and Demenais, Am J Hum Genet, 61:678, 1997) where a dominant gene was found to interact with the number of children and there was an indication of interaction, although not significant, with age at menarche. Investigation of interactions between genetic and reproductive factors in different populations exposed to different environment can have important implications for BC risk assessment.

Program Nr: 1092 from the 1999 ASHG Annual Meeting

Comparison of Genehunter 1.2 max LOD, max Heterogeneity LOD, and max NPL statistics for disease gene localization using simulated data. *C. Chen[1], D. Gordon[2], S. Finch[3], N. Mendell[3].* 1) Case Western Reserve University, Cleveland OH; 2) Rockefeller University, New York NY; 3) State University of New York at Stony Brook, Stony Brook NY.

This study compares the performance of the maximum LOD(MLOD), maximum heterogeneity LOD(MHLOD) and maximum GENEHUNTER 1.2 Nonparametric Linkage(MNPL) score for disease-gene localization. We use a simulation study considering: (i)4 disease models, (ii)100 replicates for each model, (iii) 58 pedigrees whose structure matches a field study with 592 subjects (iv)3 marker loci with 3 equally frequent alleles each, (v)marker distances of 10cM between each marker locus (vi)disease locus placed halfway between the second and third marker and (vii)either 0% unlinked families (linkage homogeneity) or 50% unlinked families (linkage heterogeneity). The disease models have: (i) a disease allele, A, with frequency 0.03, penetrance of the disease allele for those homozygous for A of 0.8, penetrance of the disease allele for those homozygous for the normal allele, B, of 0.01 and (ii)either complete dominance or complete recessivity of the disease allele. For each replicate we obtain the marker with MNPL, the marker with MLOD, and the marker with MHLOD and compute the distance of these markers from the disease locus. The MLOD (and MHLOD) are obtained by maximizing LOD (or HLOD) over penetrances, and allele frequencies, and recording the maximum of these two scores.

Comparison of distances between the location with the MLOD, MHLOD and MNPL values based on our 15-replicate pilot study indicates that (i)for data simulated under linkage homogeneity, the same location is identified by both the MLOD and MHLOD statistics; under linkage heterogeneity the location of MHLOD is much closer to the true position of the disease locus than the position of MLOD (ii) for either linkage homogeneity or heterogeneity, the position indicated by MHLOD is closer on average to the disease locus than the location identified by MNPL. Findings for the remaining 85 replicates will be presented at the meeting.

Program Nr: 1093 from the 1999 ASHG Annual Meeting

Apolipoprotein(a) polymorphisms and plasma lipoprotein(a) concentrations in Non-Hispanics Whites and Hispanics. *L. Chiu[1], R.F. Hamman[2], C.E. Aston[1], M.I. Kamboh[1].* 1) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 2) Department of Preventive Medicine and Biometrics, University of Colorado School of Medicine, Denver, CO.

Elevated levels of plasma Lipoprotein(a) [Lp(a)] are thought to be a risk factor for atherosclerosis and coronary heart disease. Plasma levels of Lp(a) are highly variable between individuals, ranging from 0.1 mg/dl to > 100 mg/dl. These levels are under strict genetic control and genetic variation in the apolipoprotein(a) [apo(a)] gene accounts for almost all variation in Lp(a) levels. In this study, we investigated the relationship between two apo(a) polymorphisms (kringle 4 and 5' pentanucleotide repeat) and plasma Lp(a) levels in normoglycemic non-Hispanic Whites (NHWs) (n=390) and Hispanics (n=214) from the San Luis Valley, Colorado. Mean (±SD) and median Lp(a) levels were 9.6±12.5 mg/dl and 3.8 mg/dl, respectively, in NHWs and 12.1±15.6 mg/dl and 4.9 mg/dl, respectively in Hispanics. The number of observed kringle 4 repeats ranged from 11 to 38 in NHWs and from 10 to 41 in Hispanics. Spearman's correlation revealed an inverse relationship between the size of the kringle 4 repeat and plasma Lp(a) levels in both populations ($r = -0.38$; $p<0.0001$ in NHWs and $r = -0.64$; $p<0.0001$ in Hispanics). About 30% and 48% of the variation in plasma Lp(a) was explained by this polymorphism in NHWs and Hispanics, respectively. This study confirms that the kringle 4 polymorphism in the apo(a) gene is a significant determinant of Lp(a) levels in both study groups. A pentanucleotide repeat polymorphism in the 5' promoter region of the apo(a) gene did not show significant impact on plasma Lp(a) levels in either NHWs or Hispanics.

Program Nr: 1094 from the 1999 ASHG Annual Meeting

Haplotype Analysis of Chemokine Receptor Polymorphisms. *V.J. Clark[1,2], R.J. Peterson[1], M. Dean[1].* 1) Laboratory of Genomic Diversity, NCI-FCRDC, Frederick, MD; 2) InterCollege Graduate Program in Genetics, Pennsylvania State University, University Park, PA.

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 and progression and the efficiency of the immune system. In addition, these proteins are important in tumorigenesis and metastasis. 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. Here, we characterize haplotypes comprising alleles at eight SNPs in the CCR2-CCR5 chemokine receptor gene region, a span of 20 kb on chromosome 3p21, using the 5' nuclease assay (TaqMan). This work is part of the NCI Cancer Genome Anatomy Project (CGAP) effort to characterize SNPs in cancer genes. Forty three-generation CEPH families, comprising 489 individuals, were screened. Haplotype states and frequencies were determined by pedigree analysis and by maximum likelihood estimation in 98 grandparents. No recombinants were needed to explain the data, yielding an upper 95% estimate of recombination at 0.004. Both pedigree analysis and maximum likelihood estimation yielded the same small number of haplotypes for which linkage disequilibrium was nearly maximal. These results suggest that maximum likelihood estimation of haplotype states and frequency, and linkage disequilibrium analysis, will be an effective strategy in the CCR2-CCR5 gene region. For genetic epidemiology studies, base line CCR2-CCR5 allele and haplotype frequencies have been determined in African American (n=30), Hispanic (n=30), and Asian (n=30) populations. As a final step, these haplotypes are being used for association analyses in case/control cohorts for lung cancer (n=271), breast cancer (n=196), AIDS-related lymphoma (n=200), and AIDS-related Kaposi's sarcoma (n=200).

Program Nr: 1095 from the 1999 ASHG Annual Meeting

HLA DQB1, DQA1, DRB1 genes and haplotypes in a normal Mexican mestizo population from Guadalajara.

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Out of 32 families living in Guadalajara, 156 healthy individuals were PCR-SSP typed. Alleles and haplotypes were counted in 54 random subjects, upon which linkage disequilibrium was estimated. Genotypes at each locus were distributed in agreement with Hardy-Weinberg expectations. Observed gene frequency (%, 2n=108) were for DQB1 (QB): *0201 (17), 0301 (16), 0302 (28), 0303 (7), 0402 (7), 0501 (18), 0502 (1), 0503 (1), 0601 (1), 0602 (5) and 0603 (1); for DQA1 (QA): *0101 (8), 0102 (3), 0103 (4), 0104 (10), 0201 (10), 0301 (30), 0401 (13), 0501 (22) and 0601 (0); for DRB1 (RB): *0101-3 (12), 0301,2 (6), 0401-11 (25), 0701,2 (12), 0801-4 (8), 0901 (2), 1001 (3), 1101-4 (8), 1201,2 (2), 1301,2 (6), 1303,4 (0), 1401,4,5 (2), 1402,3 (7), 1501,2 (4), 1601,2 (1) and other (4). Fifty-six distinct QB-QA-RB haplotypes were identified. The most commonly observed (five or more) were *0302-0301-0401/-11 (15); *0201-0201-0701/2 (8); *0501-0101-0101/3, *0301-0501-1402/3, *0402-0401-0801/4 (6); and *0301-0501-1101/4 (5). Normalized linkage disequilibrium (D') inversely correlates with the corresponding expected hapalotype frequency (F). Observed haplotypes with F>0.02 and (D')>0.5 were for QB-QA *0301-0501 (0.77), *0302-0301 (0.53), *0301-0101 (-0.80), *0501-0501 (-0.76), *0302-0201 (-0.67); for QB-RB *0202-0701/2 (0.63), *0501-0101/3 (0.81), *0302-0401/11 (0.59), *0302-0801/4 (-0.6); and for QA-RB *0301-0401/11 (0.68), *0401-0401/11 (-0.71), *0301-0101/3 (-0.74). Normalized global disequilibrium estimates were 0.55, 0.67, and 0.55 for QB-QA, QB-RB, and QA-RB, respectively. Preliminary comparisons of gene and haplotype frequency distribution with some worldwide populations indicate, as expected, that the present population is similar to other Latin American mestizo groups. Correspondence (F. Rivas): frivas@acnet.net.

Program Nr: 1096 from the 1999 ASHG Annual Meeting

Why are the majority of hereditary cases of breast cancer sporadic? A simulation study. *J. Cui, J.L. Hopper.* Centre for Genetic Epidemiology, The University of Melbourne, 200 Berkeley Street, Carlton, Victoria 3053, Australia.

Several population-based studies[1-2], including those people of Ashkenazi Jewish descent[3], have observed that 50% or more of women with breast cancer who are found to carry a germline mutation in BRCA1 or BRCA2 do not report having a family history of the disease. That is, the majority of 'hereditary' cases are 'sporadic'. Furthermore, the great majority of 'familial breast cancers' are being found not to be 'hereditary'. To investigate this phenomenon, we conducted a simulation study to evaluate the chance that a case (proband) is a mutation carrier, given the number of affected relatives. This has been undertaken for a range of plausible values of the allele frequency found in the literature (0.001 to 0.01), and the increased risk in mutation carriers relative to that in non-carriers (10 to 25, equivalent to lifetime risks of 40% to 80%, respectively). Incidence data for breast cancer in the Australian population were used to define the risk for non-carriers. Families consist of the mother, the maternal and paternal grandmothers, and sisters and aunts. The number of female siblings was generated according to a Poisson distribution, and age assigned according to a Weibull distribution fitted to the population-based Australian Breast Cancer Family Study[4]. It was found that the simulated family history distribution of cases and prevalence of mutation carriers were similar to those observed in the population-based studies. Family history of breast cancer was not a strong predictor of mutation status; as is being observed empirically in samples from the population, each affected relative increases the odds of being a mutation carrier by only 2- to 3-fold. The probability of being a mutation carrier was generally low except in families with extreme histories of breast cancer. Reference: 1. Peto J. et al. *J Natl Cancer Inst* 1999; 91: 943-949. 2. Hopper J.L. et al. Population-based estimate of the average age-specific cumulative risk of breast cancer for a defined set of protein-truncating mutations in BRCA1 and BRCA2. (submitted) 3. Hartge P. et al. *Am J Hum Genet* 1999; 64: 963-970. 4. McCredie M. et al. *Cancer Causes and Control* 1998; 9: 189-198.

Program Nr: 1097 from the 1999 ASHG Annual Meeting

MTHFR C677T mutation in parents of children with neural tube defects and in normal mestizo and native Mexican populations. *I.P. Davalos[1,2], N. Olivares[1], M.T. Castillo[1], J.M. Cantu[1], L. Sandoval[1], M.C. Moran-Moguel[1], R. Chakraborty[3], F. Rivas[1]*. 1) Genetics/Molecular Medicine Divisions, CIBO, IMSS, Guadalajara, Mexico; 2) Human Genetics Graduate Program, CUCS, UdeG, Guadalajara, Mexico; 3) University of Texas-Houston.

C677T mutation of the methylenetetrahidrofolate reductase (MTHFR) gene, associated with the thermolabile form of the enzyme, has reportedly been found increased in neural tube defects (NTD), though this association is still uncertain. A group of 107 mestizo parents of NTD children and four normal Mexican pupulations: 101 mestizo (M), 50 Huichol (H), 38 Tarahumara (T), and 21 Purepecha (P) were typed for the MTHFR C677T variant by the PCR/RFLP (HinfI) method. Twenty Caucasian (C) individuals were also studied. Genotype frequencies were in agreement with the Hardy-Weinberg expectations in all six populations. C677T allele frequency (AF, %) was 45 in the NTD group, 44 in M, 56 in H, 36 in T, 57 in P, and 35 in C. Pairwise interpopulation comparisons of AF disclosed very similar distribution between NTD and M groups (exact test, $p=0.92$). Among normal populations, differences between M and individual native groups were NS ($0.21>p>0.06$), as it was between M and C ($p=0.29$). However, very similar high frequency of the variant was found in H and P (56 and 57, $p=1.0$). With pooled data, these populations were significantly different to M and C in AF ($p<0.03$). Although genotypes were not studied in NTD affected children, similar AF in groups NTD and M does not support a causal relationship between NTD and parental MTHFR C677T genotypes. So, C677T variant cannot be regarded as a genetic risk factor for NTD in Mexican mestizo parents. Otherwise, C677T in Mexico, especially in Huichol and Purepecha natives, is very frequent as compared with other worldwide groups.
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Program Nr: 1098 from the 1999 ASHG Annual Meeting

Prediction of lung cancer cases using logistic regression and classification and regression tree models. *M. de Andrade, J.S. Barnholtz, M.R. Spitz.* UT MD Anderson Cancer Center, Dept. of Epidemiology, Houston, TX.

Recent advances in technology for identifying genetic mutations and their products have led to an interest in correlating biologic markers with epidemiological factors with a view to better understanding the natural history and etiology of diseases. There is a concomitant need for research to determine the relevant statistical methods for data analysis and interpretation. Thus a primary goal in risk characterization is to develop statistical models that reliably characterize the inter and intraindividual variability of biomarkers that predict risk for disease. Once the mechanisms of biomarker variability have been established, methods to characterize individuals into high and low risk strata can be efficiently defined so that classical epidemiological methods can be applied. Our purpose in this project is to ascertain the prognostic effect of cancer biomarkers and environmental risk factors for lung cancer through risk assessment models. To build these models, we apply two statistical methods (logistic regression and classification and regression tree). To compare these models, we use the lung cancer data from a minority study conducted at The University of Texas M. D. Anderson Cancer Center.

Program Nr: 1099 from the 1999 ASHG Annual Meeting

Effect of Biomarker Misclassification: Comparison of Two *N*-acetyltransferase 2 (*NAT2*) Genotyping Methods.

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Genotype is often used as a surrogate biomarker of phenotype in studies evaluating cancer etiology. Genotype misclassification, thus phenotype misclassification, is dependent in part, upon the specific genotype assay and may contribute to inconsistencies observed across studies. We investigated the effect of this misclassification by comparing two *NAT2* genotyping methods: a commonly used method that detects three coding region variants, *M1*, *M2* and *M3*, *vs.* one that detects all 26 reported *NAT2* alleles. Genotype data were obtained from three unpublished case-control studies, two comprised primarily of Caucasians from Poland (n=418) and Iowa (n=482) and a third (n=293) comprised of 45% African-Americans. Genotypes were used to assign individuals to rapid (R), intermediate (I), or slow (S) acetylator phenotype groups. The 3-allele method resulted in deduced phenotype misclassifications of 3.6%, 4.8% and 14%, respectively, among the three studies. Spearman correlation coefficients were 0.94, 0.91 and 0.78. Sensitivity (proportion of individuals classified as R or I phenotype by the 3-allele method relative to the 26-allele method) was 100% in each of the studies and specificities (proportion of individuals classified as S phenotype by the 3-allele method relative to the 26-allele method) were 94%, 95% and 86%. When the *M4* allele, commonly found in African Americans, was included in the third study, specificity increased to 96% and the correlation between methods increased to 0.92. Our analyses indicated that there was little difference between the two genotyping methods. However, since misclassification may affect odds ratio estimates, sample size requirements, and evaluation of gene-environment interactions, genotype-disease association studies need to consider the potential effects of genotype misclassification.

Program Nr: 1100 from the 1999 ASHG Annual Meeting

Y-chromosomes shared by descent or by state: results of a Dutch micro-geographic study. *P. de Knijff[1], T. Kraayenbrink[1], L.P. ten Kate[2], E. Bakker[1]*. 1) MGC Department of Human and Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands; 2) Department of Clinical Genetics, University Hospital Free University, Amsterdam, Netherlands.

The genetic variation at the Y chromosome was studied among 275 males from four distinct Dutch regions and a pooled random Dutch population sample. For this analysis we used 7 short-tandem-repeat (STR)-loci, 3 single-nucleotide-polymorphisms (SNP's) and an ALU ins/del polymorphism (which we, for simplicity will also include as a SNP in this abstract), all from the non-recombining part of the Y-chromosome. The 4 SNP's defined 6 distinct **Y-haplogoups** , the 7 STR's defined 145 **Y-haplotypes** and when SNP's and STR's were combined, 163 distinct Y-chromosomes could be identified. Marked differences between the regions, based on the relative frequencies of individual alleles and haplogoups/haplotypes, were found. By means of haplotype-mismatch analysis it was found that these regional differences could be atributed to a different time-scale and region of origin of a number of Y-chromosomal immigration events into the Netherlands. Keeping in mind that the regions were only separated by 50-150 km with no apparent geographic or cultural barriers, these results illustrate the power of Y-haplotypes as a tool to recognize population substructures. In addition, we paid specific attention to those STR-based haplotypes which were shared between SNP- defined haplogroups. For this purpose, we assume that any similarity between such haplotypes is more likely due to mutation processes (which we define here as identical by state, IBS) rather then because of a direct, albeit ancient, descent (designated identical by descent, IBD). Since Y-chromosome STR-based haplotypes are frequently used for forensic identification purposes it seems not irrelevant to obtain a more detailed knowledge of this phenomenon before databases containing such information could be used with confidence.

Program Nr: 1101 from the 1999 ASHG Annual Meeting

A partial central Asian/eastern Siberian origin of the Saami mtDNAs. *G.A. Denisova, M.V. Derenko, B.A. Malyarchuk.* Genetics Lab, Inst Biol Probl of the North, Magadan, Russia.

Linguistic and nuclear-gene data suggest that the Saami originated in western Siberia and extensively admixed with European populations. However, an almost completely European origin of the Saami is supported by the mtDNA studies. It was shown that the less than 6.5% of the Saami mtDNAs are members of Asian superhaplogroup M (Lahermo et al., 1996) and up to 78% of them comprise the variation of two European haplogroups V and U5 (Torroni et al., 1998). Previously, Sajantila et al. (1995) observed that the Saami are characterized by incidence (5.2%) of the mtDNA HVS I nucleotide motif 16129A-16185T-16223T-16224C-16260T-16298C. To elucidate the origin of these mtDNA sequences, we have performed an extensive survey of previously published and our unpublished data sets for this nucleotide motif. The survey of European and Asian mtDNAs revealed that among European populations this Saami nucleotide motif is characteristic with frequency <1.5% only for the Russians from northern-central European part of Russia. In spite of the absence of these mtDNAs in western Siberian populations studied (the Kets and the Samoyedic-speaking Selkups; Kazakovtseva, 1998), this Saami motif is more common in Asian populations where it shows the highest frequency (26.2%) in the Evens of eastern Siberia and the lower frequencies in the central and eastern Asia (3.9% in the Mongolians, 3.1% in the Koreans, 1.1% in the Kirghiz) and southern Siberia (2.5% in the Buryats, 5.9% in the Altaians). Even though these HVS I sequences are more common in the Saami and the Evens than in central Asians, analysis of sequence divergence appears to indicate that these mtDNA sequences harbor a much higher diversity in central Asia (0.91%) than in Siberia (0.23%) and northern Europe (0.13%). These divergence values suggest that the Mongoloid component of the Saami mitochondrial gene pool originated in a population of central Asia and expanded into northern Europe from eastern Siberia. The early Neolithic migrations of peoples from eastern Siberia to northeastern Europe are also supported by archaeological and anthropological record (Alexeeva et al., 1997).

Program Nr: 1102 from the 1999 ASHG Annual Meeting

The putative ancestral sequences to the main Mongoloid mtDNA haplogroups occur in the Buryat mitochondrial gene pool. *M.V. Derenko[1], B.A. Malyarchuk[1], I.K. Dambueva[2], G.A. Denisova[1], I.A. Zakharov[3]*. 1) Genetics Lab, Inst Biological Prob of North, Magadan, Russia; 2) Genetics Dept, Inst Experiment Biol, Ulan-Ude, Russia; 3) Genetics Lab, Vavilov Inst General Genetics, Moscow, Russia.

It has been proposed recently (Macaulay et al., 1999) that modern Eurasian mtDNA derived from the 16223 sequence (in HVSI), which, during an Upper Paleolithic expansion, gave raise to, among others, clusters A, I, M, W, X, and, after the 16223T-C/12705T-C events, all the reference-sequence-derived clusters (B, F, T, U, H, and V). The 16223T-C mutation therefore appears to be the cause of a deep split in the global phylogeny of Eurasian mtDNA. By HVSI sequencing of 40 Buryats from the South Siberia region we identified a mtDNA cluster, referred to as "16223T sequences", comprising one-forth of the Buryat lineages and occurred also in seven of the 371 Koreans, one of the 103 Mongolians and two of the 48 Havik. The sequences in this cluster are characterized by the only transition C-to-T at position 16223, and grouped near the center of the tree arguing for its ancestral position. The haplogroup status of these Buryat sequences was determined by RFLP typing for diagnostic RFLPs defining Asian and New World-specific haplogroups A, B, C, D. The RFLP identification of 16223T HVSI sequences revealed the lack of exact congruence in the RFLP-HVSI identification of those mtDNAs. The 16223T Buryat sequences were determined as a different RFLP haplotypes including C and D. One of the 16223T sequence types is characterized also by 9 bp deletion in COII-tRNALys intergenic region and presumably belongs to B haplogroup. The remaining 16223T sequences belong to "other" RFLP category. This finding appears to indicate the putative ancestral state of the 16223T HVSI sequences to haplogroups C and D at least. The 16223T sequences found in Buryats appear to be closely related to the root of the global Eurasian phylogeny, which probably lies at the 16223 node. Two 16223T Buryat sequences unclassified in the RFLP analysis but clearly non A-D and identified as "other" RFLP haplotypes therefore could be considered as a root sequence, which encompasses virtually all Eurasian mitochondrial variation.

Program Nr: 1103 from the 1999 ASHG Annual Meeting

The evolution of a variant at the *NIDDM1* locus in primates. *A. Di Rienzo[1], G. Ybazeta[1], A. Pluzhnikov[2], N. Cox[1], Y. Horikawa[3], G. Bell[3], R. Hudson[4].* 1) Dept of Human Genetics; 2) Dept. of Statistics; 3) Dept of Biochemistry & Molecular Biology; 4) Dept of Ecology & Evolution, Univ of Chicago, IL.

A variant at the *NIDDM1* locus, termed UC-SNP43, was recently proposed to account for the linkage of this region to type 2 diabetes. In pre-historical times, genetic variants contributing to diabetes susceptibility are hypothesized to confer a *thrifty* genotype allowing efficient storage of energy and fat in times of abundance and better survival during famines. Such a hypothetical selective advantage may result in patterns of variation that depart from neutrality expectations. As a step towards testing this hypothesis, we investigated the evolution of UC-SNP43 in primates. Unlike many other human polymorphisms, the non-human primates did not all share one of the two alleles found in humans (G/A). While chimpanzees, Old and New World Monkeys carried the G allele, the gorilla and orangutan carried the A allele. These results may be explained most parsimoniously by three independent mutation events. Assuming neutrality, we assessed the probability of these data given the phylogeny of primates under the general model for nucleotide substitution at a single site. The substitution rate was estimated based on the overall amount of human-chimpanzee and human-orangutan sequence divergence at this region. The probability of these data (or data with more substitutions) under neutrality turned out to be 0.011, implying that natural selection acted on this variant. One possible adaptive scenario is that positive selection drove alternative alleles to high frequency or fixation in different primate lineages. Alternatively, UC-SNP43 is an old balanced polymorphism arisen in the hominid ancestors. Under the latter scenario, great ape populations may be expected to be polymorphic at this site. We typed UC-SNP43 in a sample of 38 and 30 chromosomes from common chimpanzee and orangutan, respectively, and did not find evidence of polymorphism. Thus, if the old balanced polymorphism hypothesis is true, either one allele is at rather low frequency or different alleles were fixed in the non-human great apes. Overall, these data argue in favor of a history of non-neutral evolution for this variant.

Program Nr: 1104 from the 1999 ASHG Annual Meeting

Mitochondrial DNA variation in Ethiopian populations: evidence of regional diversification of East African groups. *B.P. Donham[1,2], T.G. Schurr[3], D.L. Donham[2], C. Panter-Brick[4], D.C. Wallace[1,2].* 1) Center for Molecular Medicine, Emory University, Atlanta, GA; 2) Department of Anthropology, Emory University, Atlanta, GA; 3) Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX; 4) Department of Anthropology, Durham University, Durham, UK.

We have characterized the mitochondrial DNA (mtDNA) variation of 157 Ethiopians by restriction fragment length polymorphism (RFLP) and control region (CR) sequence analyses. Ninety-six of these samples were collected in the Maale area, which is located in southwest Ethiopia, whereas the remaining 61 samples were collected in northern Ethiopia from several different ethnic groups. While approximately two thirds of the mtDNAs in sub-Saharan Africa belonged to macro-haplogroup L, which encompasses haplo-groups L1 and L2, this was not the case for the East African Ethiopians. Of the Ethiopian mtDNAs that belonged to macro-haplogroup L, most belonged to haplo-group L1 rather than L2. The majority of the remaining mtDNAs belonged to haplo-group L3, which lacks the HpaI 3592 site. Comparisons of the CR sequence and RFLP haplotype data also showed that several putative L3 mtDNAs are derivatives of haplogroup L1 that have lost the diagnostic Hpal 3592 site. A number of Ethiopian samples had the RFLP motif of Asian macro-haplogroup M, i.e., the DdeI 10394 and AluI 10397 sites. This result suggested that East Africa is either the source area for macro-haplogroup M mtDNAs which were subsequently spread into Asia, or that Asian mtDNAs have been introduced into Africa more recently through genetic back-flow. We also found a marked difference in the frequency of macro-haplogroups L and M in Ethiopian populations, with southern groups having 29% L and only 6% M, and the northern groups having 24% L and 26% M. These differences indicate that there may be significant regional differences in the genetic composition of Ethiopian populations.

Program Nr: 1105 from the 1999 ASHG Annual Meeting

Stroke Risk in Sibling Pairs with Sickle Cell Disease. *M.C. Driscoll[1], A. Hurlet[2], B. Berman[3], B. Files[4], L. Styles[5], K. Patel[1]*. 1) Dept Hematology/Oncology, Children's Natl Medical Ctr, Washington, DC; 2) Columbia Univ, College of P and S, NY, NY; 3) Rainbow Babies and Children's Hosp, Cleveland, OH; 4) E Carolina Univ, Greenville, NC; 5) Children's Hosp, Oakland, CA.

Cerebrovascular disease is a common complication of sickle cell disease (SCD) where 10% of patients with Hb SS have clinical stroke and another 17% have subclinical stroke by age 20. The incidence of first stroke in the pediatric SCD population is 0.7% per year, in contrast to the incidence in the pediatric non-SCD population of 0.0025%. The risk factors for clinical stroke in SCD are TIAs, acute chest syndrome, decline in hemoglobin, and increased systolic blood pressure. Although environmental risk factors for stroke in the adult non-sickle cell population are well defined, evidence for a genetic component is accumulating. A genetic risk for stroke is suggested by twin studies where the concordance for stroke is 17.7% for monozygotic twins and 3.6% for dizygotic twins and in family studies where the relative risk is increased with a parental history of stroke. This study aims to determine if there is an additional genetic risk (i.e. besides homozygosity for the sickle mutation) for stroke in a subset of patients with SCD by estimating the stroke risk in sibling pairs. The 8 pediatric centers surveyed have a SCD patient population of 3,589 patients (2235 Hb SS) less than 20 years. The incidence of stroke among the Hb SS patients is 153/2235 (6.8%). The incidence of stroke among 203 sib-pairs with Hb SS is: no stroke: 163 pairs, stroke in 1 sib: 31 pairs, stroke in both sibs: 9 pairs. Comparison of the risk for stroke in the total Hb SS group to the sib-pair group reveals an increased risk in the sib-pair group (OR = 4.2, CI 2.1-11.9, $p<0.01$). These data suggest that the relative risk for stroke in sib-pairs with SCD is higher than the overall risk for stroke in the SCD population and suggests a genetic component in a subset of SCD patients. Efforts to identify epistatic genes modifying the sickle phenotype may best be accomplished by methods currently developed for complex traits using sib-pair analysis and genome scanning or association testing for candidate genes for cerebrovascular disease.

Program Nr: 1106 from the 1999 ASHG Annual Meeting

The patterns of ancient and recent human germline mutations are similar: putatively neutral polymorphisms in human *F9* genomic sequences. *J.B. Drost, J. Feng, Q. Liu, S.S. Sommer.* Dept Molecular Genetics, City Hope Natl Medical Ctr, Duarte, CA.

The great majority of Factor IX (*F9*) mutations found in patients with hemophilia B occurred during the past 150 years. Forty-one polymorphic positions in *F9* intronic regions have been identified by restriction endonuclease fingerprint (REF) scanning of up to 20 kb in 11 randomly chosen regions of the 32 kb of genomic *F9* sequence from 84 individuals (1.538 megabases total). From these data, mutations which occurred during our species' distant past were characterized relative to an ancestral haplotype determined by analysis of seven non-human primate species. At least 21 of the mutations producing polymorphisms predate the separation of Africans and non-Africans. Thus, the mutations characterized are of very early origin. Twenty-four extant human haplotypes were identified, all of which differed dramatically from the ancestral haplotype. Recombination alone could have generated at least half of the observed haplotypes. When these ancient mutations are compared with 142 recent independent mutations from patients with hemophilia B, the pattern (transitions, transversions, and microdeletions/insertions) are similar, compatible with the hypothesis that germline mutation is due to endogenous processes that have changed little during human evolution.

Program Nr: 1107 from the 1999 ASHG Annual Meeting

French Canadian ascending genealogies: Tools for better linkage disequilibrium fine mapping. *M.-P. Dube[1], B. Brais[2], M. Jomphe[3], G.A. Rouleau[1]*. 1) 1. Centre for Research in Neuroscience, McGill University, Montreal General Hospital, 1650 Cedar Avenue, Montreal, Qc, Canada; 2) 2. Centre de recherche du CHUM-Campus Notre-Dame, 1560 Sherbrooke est, Montreal, Qc, Canada; 3) 3. Projet Balsac, Institut interuniversitaire de recherches sur les populations, Université du Québec à Chicoutimi, 555 boulvard de l'Université, Chicoutimi, Qc, G7H 2B1, Canada.

Linkage disequilibrium in founder populations provides a powerful tool for fine mapping of genes. The French Canadian population also provides ascending genealogies that can be used to complement linkage disequilibrium mapping. We report a computerized method for linkage disequilibrium fine mapping using French-Canadian ascending genealogies. The method involves two steps. First, the likely founding couple of the mutation-bearing chromosome is identified using a computerized randomization statistic. Then, using a delete-d jackknife resampling scheme, the distribution of gene mapping estimates is calculated from the count of ancestral recombinants and ancestral meioses joining the identified founding couple to the disease gene carriers. The count of ancestral meioses is estimated by randomly sampling possible disease-gene genealogies from the founding couple to the carriers. Gene mapping estimates are calculated from each marker individually, and confidence intervals of the estimates are derived from the jackknife distributions. The method, when applied to French Canadian families with oculopharyngeal muscular dystrophy (OPMD), successfully confirmed the localization of *PABP2* responsible for OPMD to a 335 kb interval on chromosome 14.

Program Nr: 1108 from the 1999 ASHG Annual Meeting

Comparison of Linkage Disequilibrium (LD) across Long Genomic Regions in Different Populations. *F. Durocher[1], A.M. Dunning[2], D. Teare[1], E. Dawson[3], S. Rhodes[3], B. Ponder[2], D. Easton[1].* 1) CRC Genetic Epidemiology Unit, Strangeways Research Centre, Cambridge, United Kingdom; 2) CRC Dept. of Oncology, Strangeways Research Centre; 3) Sanger Centre, Hinxton, UK.

The spacing of SNP markers is critical to the success and feasibility of whole genome association studies since, to give complete coverage, all the markers used will have to be in LD with their nearest neighbours. LD is affected by factors such as recombination, genetic drift, relative age of markers ans is unlikely to be uniform across the whole genome. Optimum marker spacing should reflect the strength of LD in a region. Recently founded populations have fewer haplotypes and LD may extend over longer physical distances than outbred populations or populations of small effective size and would therefore allow wider marker-spacing, thus reducing the number of genotypings necessary for a complete genome scan. The aim of our study is to draw long-range physical maps, showing the strength and extent of LD between markers, so that direct comparisons can be made between in- and out-bred populations. We are trying to identify random chromosomal regions where accurate physical maps are available over long distances and are currently working on two: Chromosome 13q12-13 and Chromosome 22q. We are initially using 8 markers on chromosome 13 over 1.3 Mb and 22 SNPs on chromosome 22, covering 0.12 Mb. We are comparing LD in these regions between the outbred East Anglian British population and the Afrikaner, the Finnish and the Ashkenazi Jewish founder populations, which have different characteristics. We are using D' to compare LD and a Monte-Carlo approach to determine significance levels and confidence regions. Initial results indicate that 5 out of 6 neighbouring pairs of markers on chromosome 22 showed significant LD, extending between markers up to 54 kb. There is also significant LD over at least 0.4 Mb of chromosome 13 but not all markers will exhibit LD with their nearest neighbours. Generally, Afrikaners have higher LD than East Anglian, but this is not always the case.

Program Nr: 1109 from the 1999 ASHG Annual Meeting

Polymorphisms in the HLA-linked olfactory receptor genes in the Hutterites. *A.C. Eklund[1], R. Raha-Chowdhury[2,3], C. Ober[1]*. 1) Human Genetics, University of Chicago, Chicago, IL; 2) Department of Pathology, Cambridge University, Cambridge, UK; 3) Department of Genetics, Yale University School of Medicine, New Haven, CT.

Genes in the MHC have been associated with mate choice and odor preferences in a variety of animals. Although the role of HLA genes in human mate choice has been controversial, studies in the Hutterites have demonstrated fewer than expected numbers of couples who match for an HLA haplotype, suggesting that in this population there is avoidance of mates with HLA haplotypes that are similar to one's own haplotype. Recently, 18 olfactory receptor (OR) genes have been mapped to the HLA region, telomeric to the HLA-F locus, providing a potential mechanism for HLA-based odor recognition and perhaps mate preferences in humans. In this study we genotyped a sample of Hutterites with diverse HLA haplotypes for polymorphisms in the HLA-linked olfactory receptor gene, FAT11. Most of the single exon in this gene (amino acids 1-251 of 316) was sequenced in DNA samples from 14 Hutterites who are homozygous for six different haplotypes. Studies in DNA representing the remaining 46 ancestral Hutterite haplotypes that occur only as heterozygotes are underway. Two single nucleotide polymorphisms were detected, both of which resulted in amino acid substitutions (Phe587Leu and Ala642Val). Phe587 and Val642 occurred together on three haplotypes (A2-Cw*1601-B51-DRB1*1301-DQB1*0603, A24-Cw*0304- B60-DRB1*1501-DQB1*0602, and A1-Cw*0602-B57- DRB1*0701-DQB1*0303), Leu587 and Ala642 occurred together on two haplotypes (A26-Cw*1203- B38-DRB1*1501-DQB1*0602 and A32-Cw*1203-B35 -DRB1*1101-DQB1*0301), and Phe587 and Ala642 occurred on a single haplotype(A3-Cw*0401-B35 -DRB1*0101-DQB1*0501).Thus at least three alleles of the FAT11 gene are present in the Hutterites. This level of variation in the FAT11 gene as well as in other OR genes in this region could contribute to the observed patterns of mate choice in the Hutterites and to individual variation in odor preferences, hypotheses that are currently under investigation. (Supported by HD21244 and GM19652).

Program Nr: 1110 from the 1999 ASHG Annual Meeting

Patterns of Genetic Diversity and Isolation by Distance. *E. Eller.* Anthropology, University of Utah, Salt Lake City, UT.

The pattern of human genetic diversity from 60 short tandem repeat (STR) loci surveyed in 15 Old World populations is consistent with a process of isolation by distance (Eller, 1999, Am. J. Phys. Anthropol. 108: 147-159). These data also show a linear decline in heterozygosity with genetic distance from Africa, consistent with an African origin of modern humans. Because STRs exhibit extremely high heterozygosity, they should be free of ascertainment bias while restriction site polymorphism (RSP) markers should be sensitive to bias since RSP markers were originally ascertained in Europeans.

Here I present the results of similar analyses from 30 RSP markers in 13 Old World populations. The main findings include the following:

1. Population differences in these markers are again consistent with a process of isolation by distance. Mantel tests show that approximately 66% of genetic distance can be explained by geographic distance. These results are similar to those from the STR data.

2. F_{ST} is 0.107. This value is similar to the STR R_{ST} value of 0.0918 and is consistent with other published values of F_{ST} and G_{ST} from a variety of genetic systems as well as craniometric data.

3. There is no excess of heterozygosity in African populations as was observed in the STR data set or an *Alu* data set (Stoneking et al. 1998, Genome Res. 7: 1061-1071). Instead, there is an excess of heterozygosity in the European populations (Northern Europeans and French) as well as in the Cambodians and the Malay. This pattern most likely is a result of ascertainment bias: these polymorphic RSP markers were first detected in Europeans. The high heterozygosity in European populations is consistent with other analyses of this data set (Jorde et al. 1995, Am. J. Hum. Genet. 57: 523-538; Rogers and Jorde 1996, Am. J. Hum. Genet. 58: 1033-1041) and with computer simulations incorporating ascertainment bias (H. Harpending, pers. comm.). These observations emphasize that we must take into account sampling bias before drawing conclusions about genetic diversity or relative effective population sizes.

Program Nr: 1111 from the 1999 ASHG Annual Meeting

Mutational diversity in inbred Middle Eastern populations. *P. Erlich[1], A. Adato[1], L. Raskin[1], H. Kalinsky[1], M. Korostishevsky[1], A. Berry[1], L. Farrer[2], B. Bonne-Tamir[1].* 1) Dept. of Human Genetics & Molecular Medicine, Tel-Aviv University, Ramat-Aviv, Israel; 2) Genetics Program, Dept. of Medicine Boston Univ. School of Medicine Boston, MA.

Rapid discovery of disease causing genes is revealing that mutations and/or haplotype profiles for various genetic disorders are often population specific. In earlier studies of Wilson Disease and Usher Syndrome, type IB in various Israeli ethnic groups (Kalinsky, et al. Hum Mut 11:45-151, 1998; Adato et. al. Am J Hum Genet 61:813-821, 1997), we documented mutations and haplotypes characteristic of each group. The Middle East is an area rich in small isolated communities and consanguineous kindreds. In these communities, it is assumed that all cases of single-gene disorders, particularly rare recessives, are the result of single ancestral founder mutations. Although families of Middle Eastern origin have proved invaluable to efforts for mapping and subsequently identifying genes for a variety of inherited conditions, several studies have demonstrated the existence of allelic and locus heterogeneity for Mendelian diseases within communties. For example, we observed two different mutations and three distinct genotypes in the ATP7B gene in an inbred Druze kindred with Wilson disease. Recently, in a highly consanguineous Israeli Palestinian kindred with non-syndromic deafness, we discovered three different coding sequence mutations in GJB2 (the connexin 26 gene). In another study of hereditary deafness in four Druze families from one village, we discovered that four different genes are responsible for the deaf phenotype: (1) the widespread 30delG mutation in GJB2, (2) a novel PDS (Pendred syndrome gene) mutation, (3) a novel OTOF mutation, and (4) an as yet unidentified gene in the fourth family. This remarkable heterogeneity may be due in part to misdiagnosis of subtly presenting syndromes as non-syndromic disorders and positive assortative mating (particularly in such instances as the deaf community). Awareness of such situations is important when considering disease gene searches or implementation of genetic screening and counseling programs in genetic isolates or inbred communities.

Program Nr: 1112 from the 1999 ASHG Annual Meeting

Heterogeneous mutations causing b-**Thalassemia in a highly inbred South Mediterranean population.** *D.M. Fathallah, R. Hafsia, M. Bejaoui, A. Hafsia, F. Amri, S. Abbes, R. Amami, MF. Ben Dridi, B. Mtimet, K. Dellagi.* The Hemoglobinopathies Study Group, Inst Pasteur de Tunis, Tunis, Tunisia.

b-Thalassemia is a single gene disorder affecting hemoglobin synthesis. It has been reported worldwide with a high incidence in countries of the Mediterranean basin, the Middle East and South Asia. In Tunisia (North Africa) a 5% frequency of b-globin morbid alleles is observed and the population is higly inbred with a consanguinity rate of 20%. The mutations producing b-Thalassemia in 45 Tunisian patients were investigated by 6 six different techniques (DGGE, PCR/ASOlabelled with P32, PCR/ASO labelled with digoxigenine, RG/PCR, PCR/Restriction analysis and direct sequencing of PCR generated b-globin specific DNA fragments). These techniques were compared and DNA sequencing was found to be the most appropriate for typing b-thalassemic patients. The spectrum of mutation uncovered was very heterogeneous as 13 different mutations were observed. 33.7% of the b-thalassemic alleles were identified as codon 39 (C to T) and 6.7% as IVS1-110 (G to A) while several mutations which had not previously been reported in the Tunisian population were observed with various incidence (4.5% for IVS1-nt 5 (G/C) frequent in population of South East Asia and 2.3% for IVS 2-nt 848 (A/C), reported almost exclusively in the Afro-American groups). Considering the six other mutations previously reported in the Tunisian patients with b-thalassemia, the total of mutations observed in this population raised to 20. The heterogeneity of the spectrum of mutations contrasts with the high inbreeding of this population. The extent of this heterogeneity could be explained either by the well documented passage of different populations over the centuries in this part of the world (Berbers, Punics, Romans,Vandals, Arabs, Black Africans, Turkish, Spanish and French) or by a high propension of the b-globin gene to spontaneously mutate or a combination of both phenomena.

Program Nr: 1113 from the 1999 ASHG Annual Meeting

A Genome-Wide Linkage Analysis in Hypertensive African American and White Siblings for Loci that Influence Variation in Pulse Pressure: The HyperGEN Network of the Family Blood Pressure Program. *K.A. Fisher[1], D.K. Arnett[1], L. Atwood[1], S. Hunt[2], H. Coon[2], M. Province[3], D.C. Rao[3], R. Myers[4], A. Oberman[5], C.E. Lewis[5].* 1) Division of Epidemiology, University of Minnesota, Minneapolis, MN; 2) Cardiovascular Genetics, University of Utah, Salt Lake City, UT; 3) Division of Biostatistics, Washington University Medical School, St. Louis, MO; 4) Boston University School of Medicine, Boston, MA; 5) Division of Preventive Medicine, University of Alabama, Birmingham, AL.

Pulse pressure (PP), an indicator of large arterial stiffness, is considered an important marker of cardiovascular health. A previously published segregation analysis indicated the presence of a major gene that influenced variability in PP in humans. Variants in the angiotensin-1 receptor gene are also reportedly associated with PP; however, limited information is available regarding whether there are genomic regions which harbor a major gene influencing PP. As part of the Hypertension Genetic Epidemiology Network, African American (AA) and white hypertensive siblings were recruited from five field centers. The mean age of the siblings was 52 in AA and 60 in whites. All were diagnosed with hypertension before the age of 60. Six seated blood pressure measurements were taken using an oscillometric method (Dinamap), and the last 5 of 6 measures were used in analysis. To reduce variability in PP (the difference between systolic and diastolic BP) contributed by non-genetic sources of variation, PP was adjusted for height, age, and physical activity. Sex-specific standardized residuals were created separately for each race group. The genome scan consisted of a 10 cM marker map (CHLC8) with 385 polymorphic, anonymous microsatellite marker loci. A multipoint linkage analysis was performed on 221 AA and 291 white sibpairs using a Haseman-Elston QTL regression analysis (MAPMAKER/SIBS). Analyses were performed separately in AAs and Whites. There was no evidence for linkage of any marker with PP. The highest lod score observed was on chromosome 19 (LOD=1.2) in AA. In this study population, little evidence was found for a genomic region with sufficiently large effect on PP.

Program Nr: 1114 from the 1999 ASHG Annual Meeting

MtDNA variation in Central Indian populations. *P. Fisher[1], K. Das[2], M.K. Das[2], P.A. Reddy[3], P.H. Reddy[3], S.S. Mastana[1]*. 1) Human Sciences Department , Loughborough University, Loughborough, Leics, UK; 2) Anthropometry and Human Genetics Unit, ISI, Calcutta, India; 3) NHGRI, NIH, Bethesda, MD, USA.

Indian population is genetically, geographically and socio-culturally very diverse. The patterns of present day genetic diversity in India offer important clues to the evolutionary history of human populations. Caste structure has played an important role in shaping up the observed genetic variation. Therefore it is challenging to analyse and document patterns of genetic diversity. We have surveyed mtDNA sequence variation among Eight Central Indian caste and tribal populations using PCR amplification and sequencing of HVR1 region. Estimates of mtDNA sequence diversity ranged from 0.004 to 0.028 and G_{ST} estimates among castes groups were lower than tribal populations. Comparisons are made with mtDNA haplotypes from Caucasian, Asian, African and other Indian populations to assess relative position of these populations.

Program Nr: 1115 from the 1999 ASHG Annual Meeting

Linkage Disequilibrium study on Nonsyndromic Cleft Lip/Palate on three racial heterogeneous populations from Colombia. *I. Fonseca[1], T. Restrepo[1], N. Rojas[2], A. Mario[2], C. Isaza[3], M. Arcos-Burgos[1]*. 1) Biology, Universidad de Antioquia, Medelln, Colombia; 2) Smile operation, Colombia; 3) Universidad del Valle, Cali, Colombia.

In order to compare racial effects on predisposition to develop Nonsyndromic Cleft Lip/Palate (CLP), we have ascertained pedigrees and cases and controls from three different regions from Colombia, Antioquia (100 families), Cundinamarca (170 cases) and Valle (119 cases) characterized by its different racial components, Caucasoid, Amerindian and Negroid, respectively. In all of them could be determined, by using the unified model of complex segregation, that only a major gene was able to explain the predisposition to CLP. Preliminary linkage disequilibrium analysis by using the exact test Linkage disequilibrium analysis on 6p21.3-21.5 with the microsatellite markers D6S272, D6S1019, D6S1006, D6S1959 shows that strong Hardy-Weinberg disequilibrium is observed only at the marker D6S1959 ($p<0.0001$) due to an increasing number of the heterozigotes ($f=0.44$). In other studies, the genomic region surrounding this marker has shown linkage to CLP. We think that increasing the resolution of this area mapping by using admixture linkage disequilibrium (MALD) could be performed.

Program Nr: 1116 from the 1999 ASHG Annual Meeting

A novel founder mutation in variegate porphyria patients from Chile and its future implications. *J. Frank[1,4], V.M. Aita[2], W. Ahmad[1], H. Lam[1], C. Wolff[3], A.M. Christiano[1,2].* 1) Dept. of Dermatology, Columbia University, New York, NY; 2) Dept. of Genetics and Development, Columbia University, New York, NY; 3) Dept. of Medicine, University of Chile, Santiago, Chile; 4) Dept. of Dermatology, University Clinic of the RWTH Aachen, Aachen, Germany.

Variegate porphyria (VP) (OMIM 176200) results from a partial deficiency in the activity of protoporphyrinogen oxidase (PPO), the penultimate enzyme of the porphyrin-heme biosynthetic pathway. The disease is usually inherited in an autosomal dominant fashion with incomplete penetrance. Clinically, VP is characterized by cutaneous symptoms on the sun exposed areas of the skin and by acute neurovisceral attacks which can lead to coma and death. In an effort to characterize the molecular basis of VP in South America, we studied 8 of 21 reported VP families from Chile by PCR, heteroduplex analysis, automated sequencing, restriction enzyme digestion and haplotyping analysis. We detected 3 different mutations, 1239delTACAC, R168H, and 1330delT. Interestingly, 1239delTACAC, which results in a frameshift and a downstream premature termination codon, was found in patients from 4 unrelated families, living in different parts of Chile. Therefore, we performed haplotype analysis using 15 microsatellite markers which closely flank the PPO gene on chromosome 1q22, in the order ptel-D1S196-ATA38A05-D1S426-D1S1677-D1S104-D1S1679-D1S2705- D1S484-D1S2707-D1S398-D1S1653-D1S1600-D1S1595-D1S303-D1S2140-pcen. This interval spans approximately 21 cM on the Genethon linkage panel, indicating that these 15 markers and the PPO gene are tightly linked. Haplotype analysis revealed the occurrence of 1239delTACAC on the same chromosome 1 haplotype in 11 mutation carriers from 4 unrelated families with VP, consistent with 1239delTACAC representing a founder mutation for VP. These findings comprise the first genetic studies of the porphyrias in South America, and will streamline and facilitate the elucidation of the molecular basis of VP in Chile as well as in its neighboring countries Argentina, Peru and Bolivia by first screening for the mutation 1239delTACAC.

Program Nr: 1117 from the 1999 ASHG Annual Meeting

Molecular reconstruction of the genealogy of the isolated population of the Costa Rican Central Valley. *N.B. Freimer[1], R. Ophoff[1], D. Meshi[1], W. Poon[1], P. Oefner[2], S. Service[1]*. 1) Dept Psychiatry, LPPI, Univ California, San Francisco, CA; 2) Department of Biochemistry, Stanford University, Palo Alto, CA.

The potential value of homogenous isolated populations in genetic mapping studies is increasingly clear. However, genetic evidence supporting the homogeneity of particular isolates is usually lacking. The development of population-specific Y-chromosome polymorphisms provides an important tool for reconstructing the genealogy of a population at the molecular level. We are using the isolated population of the Costa Rican Central Velley for several mapping studies of complex traits, such as severe bipolar mood disorder. It is thought that this population is mainly descended from the admixture between approximately 80 Spanish settles and a small group of indigenous Amerindians, primarily in the 17th and 18th centuries. This admixture was followed by substantial population growth in isolation, with minimal new immigration. To study the molecular genealogy of this population we genotyped individuals (bipolar patients and controls) sampled from the Central Valley at an Amerindian-specific SNP (DYS199) and 10 microsatellite markers located in the non-recombining regions of the Y chromosome. Testing mitochondrial polymorphisms in these individuals completed the molecular genealogical study. We will compare the molecular homogeneity in patients versus controls and discuss the implications for association studies in isolated populations.

Program Nr: 1118 from the 1999 ASHG Annual Meeting

Longevity in Iceland: Fertility and Genetics. *M.L. Frigge[1], D.F. Gudbjartsson[1,2], H. Gudmundsson[1], K. Stefánsson[1].* 1) deCODE genetics, INC., Reykjavík, Iceland; 2) Institute of Statistics and Decision Sciences, Duke University, Durham, NC.

An article recently appeared (Westendorp and Kirkwood, Nature, 1998) in which the authors analyzed the relationship between longevity and reproductive success in the British aristocracy during the period from 740 to 1875 AD. They report that in that population longer lived women both have fewer children and start having children later than women who die younger. They suggest that this provides evidence for the first time in a human population that there is a trade-off between reproduction and the somatic maintenance needed for longevity. Numerous newspaper articles have interpreted this as suggesting that if women want to live longer they should hold off having children. Having access to a unique genealogical database of all Icelanders from the present back to the founding of the country in 870 AD, there was interest in determining whether the pattern observed for a small number of British aristocracy would hold up for the entire modern population history of Iceland -- about 750,000 persons, of which 275,000 are currently alive. Iceland is an ideal population for this study since the population is quite homogeneous with respect to many conditions which might otherwise confound such a study of longevity in other European populations during this time period. Here evidence is reported that Icelandic women who live longer do not have fewer children on average than those who die younger. Nor do longer lived Icelandic women begin having children later in life. Similar results are presented for Icelandic men. The genealogical database also provides a singular opportunity to investigate the familial clustering of longevity in an entire human population, and thus to assess the genetic component of longevity in Iceland. Traditional measures of relatedness, such as recurrence risk (1.8 for sibs, 1.3 for cousins) are presented along with some new measures designed to take advantage of the whole population genealogy.

Program Nr: 1119 from the 1999 ASHG Annual Meeting

Rheumatoid Arthritis Severity and Dosage of the Shared Epitope: Results of a Meta-Analysis. *J.D. Gorman[1], J.J. Chen[2], G. Thomson[3], M.E. Suarez-Almazor[4], L.A. Criswell[1]*. 1) Division of Rheumatology, University of California, SF; 2) School of Public Health, Saint Louis University, St. Louis, MO; 3) Department of Integrative Biology, University of California, Berkeley, CA; 4) Department of Public Health Services, University of Alberta, Edmonton, AB, CANADA.

Objective:To evaluate by meta-analysis the risk of severe RA in relation to dosage of the HLA-DRb1 shared epitope (SE). **Methods**: A MEDLINE search and hand searches of 6 journals were conducted for studies with molecular typing and 1 or more of 6 desired outcomes. Authors were contacted for incomplete data, and all were asked for original data sets, with covariate information. Woolf's weighted ORs were pooled across studies and tested for homogeneity. When possible, ethnic and clinical subgroup analyses were performed. **Results**: 41 studies met inclusion criteria (5433 patients, 17 countries, 5 continents). ORs for the association of 1 SE copy (OR1) and 2 SE copies (OR2) with each outcome are shown below (*reference group=0 SE alleles). A strong dosage effect of the SE was apparent only for joint surgery [OR for 2 vs.1 SE copies=2.1 (1.3-3.4)]. Breslow-Day homogenity tests indicated substantial heterogeneity across studies for all outcomes except surgery, partly explained by ethnic variation.

	RF+	RA Course	Erosions	Surgery	Nodules	Other EAMs
OR1*/95%CI	1.8/1.4-2.4	1.5/1.0-2.2	1.8/1.4-2.2	1.8/1.0-3.3	1.5/1.2-2.0	1.2/0.8-1.8
OR2*/95%CI	2.6/1.8-3.8	1.7/1.0-2.8	2.2/1.6-2.9	3.7/1.9-7.4	1.7/1.3-2.3	1.8/1.1-2.8

Conclusions: Results of this meta-analysis demonstrate a dose-dependent increased risk for surgery. Although this was not observed for other outcomes, caution should be exercised given their heterogeneity. Further identification of important sources of heterogeneity will require thorough examination of covariate effects.

Program Nr: 1120 from the 1999 ASHG Annual Meeting

The inheritance of Rheumatoid Arthritis in Iceland. *S.F.A. Grant[1], M. Frigge[1], J. Thorsteinsson[2], B. Gunnlaugsdottir[2], A. Geirsson[2], A. Vikingsson[2], K. Erlendsson[1], D. Oskarsson[1], A. Kong[1], J. Gulcher[1], K. Stefansson[1], K. Steinsson[2]*. 1) Decode Genetics, Reykjavik, Iceland; 2) Dept of Rheumatology and Center for Rheumatology Research, National Hospital, Reykjavik, Iceland.

Rheumatoid Arthritis (RA) is the commonest form of inflammatory arthritis, affecting approximately 1% of the world's population. Although there is a large body of evidence showing that RA is immune mediated, the etiology remains unresolved. From animal models, a genetic component is believed to influence the onset and severity of the disease. In humans it has long been suggested that variation in genes encoded in the HLA region accounts for approximately one third of the genetic component in RA, with other studies suggesting that additional loci contribute to the disorder. Twin studies have shown a concordance rate between 15 and 30% whilst the prevalence ratio for siblings of RA patients is approximately 5. However, these findings should be regarded with caution as studies in small proportions of a heterogeneous population are susceptible to sampling error and selection bias.

We have developed a population-based computerized genealogy database to examine multigenerational relationships amongst individuals in the homogeneous population of Iceland. Using an algorithm, the Minimum Founder Test (MFT), the least number of founders accounting for our RA patient list was compared with 500 sets of same sized control groups. A significantly fewer number of founders are necessary to account for our patient list than for the random sets of matched controls ($p<10^{-8}$) where average pair-wise IBD sharing for patients is greater than 6 standard deviations higher. In addition, the general risk of developing RA is 1.07% and the recurrence risk for siblings is ls = 4.41.

This data confirms that there is a significant genetic component to RA.

Program Nr: 1121 from the 1999 ASHG Annual Meeting

Head injury, APOE genotype, and familial risk of Alzheimer disease. *Z. Guo[1], L.A. Cupples[1], J.L. Haines[2], L.A. Farrer[1], for the MIRAGE Study[1]*. 1) Boston University School of Medicine, Boston, MA; 2) Vanderbilt University School of Medicine, Nashville, TN.

Some studies have suggested that head injury is a risk factor for Alzheimer disease (AD), and that this risk is heightened among carriers of the apolipoprotein E (APOE) e4 allele. We examined the effects of head injury and APOE genotype on AD risk in a large family study. A total of 2,233 probands who met criteria for probable or definite AD and their 14,668 family members (4,465 parents, 7,694 siblings, and 2,509 spouses) were ascertained at 13 centers in the U.S., Canada and Germany. Conditional logistic regression comparing probands with their unaffected spouses yielded odds ratios for AD of 9.9 (95% CI = 6.5-15.1) for head injury with loss of consciousness and 3.1 (2.3-4.0) for head injury without loss of consciousness. Survival analysis showed that at age 93 years the cumulative risk of AD among parents and siblings was 77±7% for those with head injury and 34±1% for those without head injury. Similar patterns were observed in spouses, although the total risk was low. Among parents and siblings, the relative risk of AD was 4.0 (2.9-5.5) for head injury with loss of consciousness and 2.0 (1.5-2.7) for head injury without loss of consciousness. Among spouses, the relative risk of AD was 6.1 (2.6-13.9) for head injury with loss of consciousness and 1.3 (0.4-4.1) for head injury without loss of consciousness. The joint effects of head injury and APOE genotype were evaluated in a sub-sample of 942 probands and 327 controls (spouses and siblings). Among e4 homozygotes, the odds of AD was 10.3 (1.6-65.3) for those with head injury and 7.9 (4.3-14.3) for those without head injury, compared to those lacking both factors. Among e4 heterozygotes, the odds of AD was 5.7 (3.1-10.5) for those with head injury and 3.1 (2.1-4.7) for those without head injury. Head injury in the absence of e4 increased AD risk 3.3 times (2.0-5.5). We conclude that head injury is a risk factor for AD independent of APOE e4. Although there is no significant interaction between head injury and a family history, the lifetime risk of AD is extremely high among first-degree relatives with a prior head injury.

Program Nr: 1122 from the 1999 ASHG Annual Meeting

High prevalence of complement C7 deficiency among healthy blood donors of Morrocon Jewish ancestry. *D. Halle[1], A. Sasson[1], D. Elstein[1], M. Schlesinger[2], A. Zimran[1]*. 1) Shaare Zedek Medical Center , Jerusalem, Israel; 2) Barzilai Medical Center, Ashkelon, Israel.

It is well recognized that the cytolytic acticity of the complement system is critical for resistance to Neisseria meningitidis. More than a decade ago we initiated a study to assess the prevalence of terminal complement deficiencies among survivors of sporadic meningococcal disease in Israel (QJM, 240:349-358, 1987). We uncovered an unexpectedly high prevalence of C7 and C8 deficiency among subjects of Moroccan Jewish ancestry, but not a single case among the subjects of Ashkenazi Jewish ancestry although the latter represents the largest ethnic group both in Israel and hence, in our series. Cloning of the complement component genes in recent years, has led to identification of the mutation in the Moroccan population, initially via RFLP and then via sequencing, which is G357R. In the current study, PSM-PCR site-directed mutagenesis was used in conjunction with designer primers in order to locate the mutation specific to our Moroccan population. We describe a new method using the restriction enzyme Mlu 1 to cut the mutant allele and Dde 1 to cut the normal allele. The advantages of this protocol include simplicity of design, completion within hours, accuracy, and it is inexpensive. In all, 284 healthy subjects (568 alleles) who were of Moroccan ancestry on both sides were screened. Five heterozygotes were uncovered, giving an allele frequency of approximately 0.9% in this ethnic group. Although there have been a few studies which suggest beneficial effects of terminal complement deficiencies (e.g. protection against autoimmune diseases, multiple sclerosis), these theories have not been extrapolated to Jewish populations. With the availability of this method, larger screens may be attempted and the issues of gene frequency and selective advantage addressed.

Program Nr: 1123 from the 1999 ASHG Annual Meeting

Familialty of quantitative traits derived during an oral glucose tolerance test in normoglycaemic relatives of one type 2 diabetic parent. *T. Hansen[1], C. Ekstroem[1], S.A. Urhammer[1], H. Eiberg[2], J.J. Holst[2], O. Pedersen[1]*. 1) Steno Diabetes Center, Gentofte, Denmark; 2) University of Copenhagen, Denmark.

The present study was undertaken in order to describe the familiality of quantitative traits which could be obtained during an oral glucose tolerance test (OGTT). Sixty families containing 224 offspring and 32 spouses of type 2 diabetic parents were examined by a 4 hour 75g OGTT. Plasma glucose, s-insulin, s-C-peptide levels were analysed at 18 time points, and p-GIP (glucose dependent insulinotropic polypeptide) and p-GLP-1 (glucagon like peptide 1) were analysed at 12 time points during the OGTT. Familiality (h2) of the examined traits was estimated as the ratio of the additive genetic variance to the total phenotypic variance using a variance component model and adjusting for body mass index, age and gender. Familiality of each trait was examined 1) in the fasting state, 2) as incremental area under the curve (AUC) from 0-120 min and from 0-240 min 3) and as the highest h2 obtained for a single time point.

h2 (SE)	Glucose	Insulin	C-peptide	GIP	GLP-1
Fasting	0.71 (0.15)	0.35 (0.22)	0.56 (0.15)	0.67 (0.21)	0.91 (0.32)
AUC 0-120	0.39 (0.24)	0.19 (0.25)	0.54 (0.22)	0.28 (0.22)	0.75 (0.23)
AUC 0-240	0.40 (0.24)	0.21 (0.25)	0.54 (0.22)	0.24 (0.22)	0.77 (0.15)
Peak	0.56 (0.24)	0.46 (0.24)	0.47 (0.23)	0.46 (0.25)	0.57 (0.15)
	(140 min)	(10 min)	(160 min)	(90 min)	(90 min)

In conclusion, several quantitative traits obtained during an OGTT are highly familial, and will be used to search for prediabetic quantitative trait loci.

Program Nr: 1124 from the 1999 ASHG Annual Meeting

Characterization of b**-thalassemia mutations in Armenia.** *K. Harutyunyan[1], V. Surin[2], H. Youssoufian[3], V.M. Der Kaloustian[4]*. 1) Institute of Molecular Biology, National Academy of Sciences, Yerevan, Armenia; 2) Center of Hematology, Academy of Medical Sciences, Moscow, Russia; 3) Departments of Molecular and Human Genetics and Medicine, Baylor College of Medicine, Houston, Texas, U.S.A; 4) Departments of Pediatrics and Human Genetics, McGill University, Montreal, Quebec, Canada.

Armenia is one of the countries of the former Soviet Union situated in the region of the Caucasus. Its population is around 3.5 million, composed more than 95% of Armenians, but also of Yezidis, Kurds and Russians. Armenians are recognized as a separate ethnic group since 750 B.C.

To-date, 12 families with members suffering from b-thalassemia (b° and b^+ forms) have been registered in the country. We have performed mutation analysis by direct sequencing of the PCR fragments of the b-globin genes of two unrelated Armenian patients. Three mutations were identified. CD15 (TGG-TAG),IVS1,nt(T-C), and CD8(-AA). The haplotype of these patients have been determined. The population studies for the gene frequency of b-thalassemia in Armenia, as well as the characterization of the mutations in the other known patients are in progress. Although these mutations have been previously found in patients with other ethnic backgrounds, we are reporting them for the first time in Armenians. (This work was supported in part by a grant from the Armenian branch of the Open Society Institute).

Program Nr: 1125 from the 1999 ASHG Annual Meeting

Prevalence of Factor V Leiden Mutation in Greek Patients with Homozygous Sickle Cell Anemia or Double Heterozygous Sickle Cell/ Thalassemia. *T. Hatzis[1], K. Makatsoris[2,3], M. Drosou[5], C. Pantos[3], V. Malliopoulou[3], T. Antoniadi[2], M. Gavalaki[4], E. Economou-Petersen[4], M.B. Petersen[2], H. Karageorgiou[3].* 1) Haematology, "Mitera" Maternity Hospital, Athens, Attica, Greece,; 2) Department of Genetics and Molecular Biology, "Mitera" Maternity and Surgical Center, Athens, Greece; 3) Department of Pharmacology, University of Athens Medical School; 4) Thalassemia Treatment Unit, Drakopoulion Blood Bank Center; 5) Thalassemia Treatment Unit, Piraeus General Hospital, Athens, Greeceâ.

In Caucasians the G1691A mutation of the factor V gene (FV Leiden) creates increased risk for thromboembolism. Homozygous Sickle Cell Anemia (S/S) or double heterozygous Sickle Cell/ Thalassemia (S/thal) patients suffer frequent and recurrent thromboembolic events, whose cause has not yet been identified as a single risk factor. The aim of this study was to estimate the FV Leiden prevalence in Greek patients with homozygous Sickle Cell Anemia or double heterozygous Sickle Cell/ Thalassemia. Results on a population of 29 patients are shown here and are compared with the results of a study among healthy Greek blood donors, as previously determined: 29 patients with (S/S) or (S/thal):allele frequency 10.3%, carrier rate 17.1%. 160 healthy Greek blood donors: allele frequency 2.5%, carrier rate 5%. Conclusions: although the population studied is small, these results indicate a contradiction to previous similar studies in other populations, that have shown smaller carrier rates among S/S and S/thal patients and suggested an obvious beneficial advantage when this thrombogenic mutation is absent in people who have many other survival risks since birth.

Program Nr: 1126 from the 1999 ASHG Annual Meeting

Concordance in geographic patterning of ancient and modern Amerindian mtDNA variation. *M.G. Hayes, S.W. Carlyle, D.H. O'Rourke.* Department of Anthropology, University of Utah, Salt Lake City, UT.

We report original research on ancient DNA variation in three geographic regions, the North American Arctic, the eastern Great Basin, and the US Southwest, as well as published data on aDNA variation in the western Great Basin (Kaestle 1998) and the US Midwest (Stone and Stoneking 1998). Comparisons of these ancient samples to contemporary American Indian populations inhabiting the same geographic locales demonstrates concordance in the geographic patterns of variability over time. At least in North America, the observed mtDNA geographic patterns are of considerable antiquity and have been stable for at least the past two to four millenia. The aDNA data from this diverse collection of archaeological samples indicate that all major mtDNA lineages characterizing modern Amerindian populations were present prehistorically. These results are most obvious in examination of mtDNA haplogroup frequencies based on RSP and length polymorphisms, but are also consistent with the limited sequence data available from ancient samples. While fewer ancient South American samples have been molecularly characterized, the correspondence between ancient populations and contemporary groups appears to hold. These results indicate that 1) early colonists to the Americas possessed considerable mtDNA diversity, weakening claims for a reduction in Amerindian molecular variation as a result of a founding bottleneck; 2) population substructure in Amerinds is of considerable antiquity and has been relatively stable for long periods of time; and 3) regional patterns of molecular variability were essentially unaffected by population declines at contact or later non-Amerindian admixture. This work was supported by grants from the Physical Anthropology, Archaeology, and Arctic Social Science programs of the National Science Foundation, the Wenner-Gren Foundation for Anthropological Research, and the University of Utah.

Program Nr: 1127 from the 1999 ASHG Annual Meeting

Common paraoxonase gene variants, mortality risk and fatal cardiovascular events in elderly subjects. *B.T. Heijmans[1,2], R.G.J. Westendorp[2], C. Kluft[1], A.M. Lagaay[2], D.L. Knook[1,2], P.E. Slagboom[1].* 1) Gaubius Laboratory, TNO Prevention and Health, Leiden, The Netherlands; 2) Section of Gerontology and Geriatrics, Department of General Internal Medicine, Leiden University Medical Center, Leiden, The Netherlands.

The enzyme paraoxonase may be an important modulator of cardiovascular disease risk because of its ability to protect LDL from oxidation. We examined whether two functional variants of the paraoxonase gene (Met-55/Leu and Gln-192/Arg) were associated with all-cause mortality and fatal cardiovascular events. This was done within a population-based study among subjects aged 85 years and over in a cross-sectional and a prospective design.

In the cross-sectional analysis the distribution of both paraoxonase genotypes was similar in the subset of 364 elderly subjects who were born in Leiden, The Netherlands, as compared to 250 young subjects whose families originated from the same geographical region. The polymorphisms were in linkage disequilibrium ($P<0.00001$). The rare Arg-allele almost always occurred in combination with the frequent Leu-allele, which gives rise to a common haplotype carrying both putative risk-alleles. The frequency of this haplotype was similar in elderly and young subjects (0.313 versus 0.284, respectively).

The complete cohort of 666 elderly subjects was followed over 10 years. The risk of all-cause and cardiovascular mortality were not increased in elderly subjects with the paraoxonase Leu/Leu (RR, 1.1 [95% CI, 0.9-1.5] and 1.3 [95% CI, 0.8-2.0], respectively) or the Arg/Arg genotype (RR, 0.9 [95% CI, 0.7-1.2] and 0.7 [95% CI, 0.4-1.3], respectively). In a subset of patients with diabetes, the all-cause mortality risk was nonsignificantly increased in Arg/Arg carriers.

Paraoxonase gene variants, previously associated with coronary artery disease, are not likely to have a major effect on the risk of fatal cardiovascular disease in the population at large. Adverse effects of the gene variants might be observed in subjects exposed to factors that enhance oxidative stress such as diabetes.

Program Nr: 1128 from the 1999 ASHG Annual Meeting

Familial clustering of myocardial infarction in the Icelandic population: Evidence for genetic components. *A. Helgadottir[1], H. Petursson[1], A. Kong[1,2], J. Gulcher[1], M. Frigge[1], V. Gudnason[3], K. Stefansson[1].* 1) deCODE Genetics Inc, Reykjavik, Iceland; 2) Departments of Human Genetics and Statistics, University of Chicago; 3) Icelandic Heart Association Heart Preventive Clinic, Reykjavik, Iceland.

Many studies have shown that coronary heart disease and myocardial infarction (MI) aggregate within families. All of them have included relatively small proportions of populations. This is a population-based study which determines the significance of familial clustering among Icelandic MI patients. We took advantage of our population-based genealogical database, to study how 6860 MI patients cluster into families. Data regarding the study group were collected between 1981 and 1995, from all MI sufferers in Iceland, by the Icelandic Heart Association as part of the World Health Organization MONICA Project. Control groups were created from the genealogy database in such a way that every person in the study group corresponded to one person in the control group, born in the same year. We have developed a tool called the Minimum Founder Test which compares minimum number of ancestors required to account for the group of patients with the minimum number of ancestors required to account for the control groups, at different timepoints in the past. Going back to the years between 1800 and 1900 the minimum number of founders representing the group of patients were significantly fewer than those representing the control groups. The kinship coefficient for the myocardial infarction patients was 1.7 10-4 compared to an average kinship coefficient, for 500 control groups (each consisting of 7466 individuals), of 1.6 10-4 (SD: 2.4 10-6). The relative risk for first-, second-, and third degree relatives were 1.4, 1.2 and 1.1, respectively. The risk of early onset MI for siblings and first cousins of patients with early-onset MI were 3.5 and 1.7, respectively. This study is the first population-based study using an extensive genealogy database examining the genetic contribution to MI. Our study encourages the search for genes responsible for increased susceptibility to MI in the Icelandic population.

Program Nr: 1129 from the 1999 ASHG Annual Meeting

The relationship of Lp(a) levels with coronary artery disease is genotype dependent for three apo(a) gene polymorphisms in Asian Indians in Singapore. *C.K. Heng[1], W.F.K Ho[1], N. Saha[2], M.C. Tong[3], Y.S. Tan[3], M.I. Kamboh[2]*. 1) Dept Pediatrics, National Univ Singapore, Singapore, Singapore; 2) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, U.S.A; 3) Singapore Heart Centre, Singapore General Hospital, Singapore,Singapore,Singapore.

In this study, we genotyped the pentanucleotide (TTTTA), +93 and kringle-4 (K4) sites of the apo(a) gene in Asian Indians residing in Singapore. The study subjects consisted of 226 angiographically confirmed CAD patients and 161 healthy age-matched controls. We used the logistic regression model with genotype data and Lp(a) as predictors and CAD status (with or without the disease) as the dependent variable. Probability curves for CAD occurrence was plotted against Lp(a) concentrations. Characteristic sigmoid curves associated with each genotype were obtained. The predicted probabilities of CAD susceptibility for two groups of K4 pooled genotypes were similar at Lp(a) concentration of < 1mg/dl but those with >24 repeats had their probabilities increased at a higher rate than those with <24 repeats, along with increasing Lp(a) level. For TTTTA, the probability curves criss-crossed at 13mg/dl for the 2 pooled genotypes. Below 13 mg/dl, the risk of CAD was higher for those with <8 repeats and vice versa beyond 13 mg/dl. The +93 genotypes showed a very distinctive trend with the *CT/TT* individuals at higher risk of CAD compared to the common *CC* genotype across the whole range of Lp(a) levels. Our findings underscored the complex web of relationships between risk factors and genetic variations when used to predict CAD risk. Current analytical methods could only demonstrate such complex relationships crudely by means of separate CAD probability plots of each genotype and its associated intermediate trait. The overall CAD risk as a function of the relationships between many predictive genes and intermediate traits could not be elucidated with such simplistic representations. Insightful revelations would be obtained only when more advanced models that could integrate all the relevant predictors are developed.

Program Nr: 1130 from the 1999 ASHG Annual Meeting

Global correlations between gene frequencies and disease rates. *S.R. Hirth[1], N.J. Schork[1,2,3,4,5]*. 1) Epidemiology and Biostatistics, Case Western Reserve U., Cleveland, OH; 2) Program for Population Genetics, Department of Biostatistics, Harvard School of Public Health, Boston, MA; 3) The Jackson Laboratory, Bar Harbor, ME; 4) The Genset Corporation, La Jolla, CA; 5) Genset, SA, Paris, France.

Ecological studies assessing the relationship between environmental factors and disease frequencies across populations have been a popular and useful way of investigating initial hypotheses about the etiology of several common disorders. In recent years, population genetics studies have also been carried out for many of these same global populations, providing the opportunity to link gene frequencies to such ecological studies. We have searched publicly available databases for situations where all three types of information are available for the same population. For such cases, we then combined population gene frequencies for several commonly typed polymorphisms with the available data on disease rates and environmental exposures, by population, to investigate possible correlations. We present results for several common disorders for which information is available, including the correlation between HLA gene frequencies and tuberculosis across global populations. Further, we describe how studies such as these can be a valuable source of information regarding the design and conduct of public health initiatives and research programs.

Program Nr: 1131 from the 1999 ASHG Annual Meeting

A measure of ambiguity in SNPs in presence of linkage disequilibrium. *S.E. Hodge, J. Hoh.* Columbia Univ, New York, NY.

The extent of haplotype ambiguity in a string of single nucleotide polymorphisms (SNPs) was quantified by Hodge et al. (Nat Genet 21: 360, 1999). In their measure, the level of ambiguity increases with increasing numbers of loci and as loci become more polymorphic. That work assumed linkage equilibrium (LE). However, linkage disequilibrium (LD) provides additional information about the haplotypes at a site, thereby diluting the level of ambiguity. The ambiguity vanishes altogether when LD reaches its maximum value. Here, we extend the ambiguity measure (f) to allow for LD between each successive pair of SNPs. We derive the formula f= 4yz, where x, y, z, and w are the frequencies of the ++, +-, -+, and -- haplotypes, respectively, and w.l.o.g. xw > yz. Alternatively, f can be expressed in terms of the allele frequencies and the LD parameter d. We also extend the formula to triads of two parents plus one child. In genome-wide LD studies to map common disease genes, a dense map of SNPs has been utilized to detect association between a marker and disease. Therefore, the measurement of ambiguity can help investigators to determine a more efficient map, designed to minimize ambiguity and subsequent information loss. We calculate our measure for relevant SNPs in the published LPL dataset (Clark et al., AJHG 63: 595, 1998; Nickerson et al., Nat Genet 19: 233, 1998), obtaining values ranging from a low of 0 to a high of 11%, in that particular dataset.

Program Nr: 1132 from the 1999 ASHG Annual Meeting

Lactase haplotype diversity in the Old World. *E.J. Hollox[1], M. Poulter[1], M. Zvarik[2], V. Ferak[2], A. Krause[3], T. Jenkins[3], N. Saha[4], A. Kozlov[5], D.M. Swallow[1].* 1) MRC Human Biochemical Genetics Unit, Galton Laboratory, University College London, London, UK; 2) Department of Molecular Biology, Comenius University, Bratislava, Slovakia; 3) Department of Human Genetics, University of the Witwatersrand, Johannesburg, Republic of South Africa; 4) Department of Human Genetics, University of Pittsburgh, USA; 5) ArctAn-C Innovative Research Laboratory, Moscow, Russia.

Lactase persistence is a genetic trait present at different frequencies in different populations: it is frequent in northern Europeans and certain African and Arabian nomadic tribes, groups that have a history of drinking fresh milk. Selection is likely to have had an important role in the establishment of these varying frequencies. We have previously shown that the lactase persistence/non-persistence polymorphism in humans is *cis*-acting to the lactase gene and that lactase persistence is associated, in Europeans, with the most common 70kb lactase haplotype, termed **A** (Harvey et al. Ann. Hum. Genet. 62,215-223 1998). We have studied polymorphisms in over 1000 chromosomes to examine 11-site haplotype frequencies in 11 populations that differ in lactase persistence frequency. Our data show that there are four common haplotypes (**A,B,C,U**) that are not closely related, having a minimum of three sites different from each other. These four haplotypes account for over 80% of all haplotypes in non-African populations, but only account for 48% of all haplotypes in sub-Saharan African populations. The **A** haplotype is shown to be at a much higher frequency in northern Europeans, where persistence is common, as compared to other populations where persistence is rare. The **U** haplotype has an unusual distribution, being common in East Asia and sub-Saharan African populations but only observed in two individuals from Indo-European populations. Analysis of 70kb haplotypes is small enough to observe linkage disequilibrium yet large enough to observe recombinational events. A model of the relationship between haplotypes suggests generation of diversity by point mutations and recombinations, probably including an ancient recombination within a region rich in repetitive elements.

Program Nr: 1133 from the 1999 ASHG Annual Meeting

Phylogenetic networks: search for traces of ancestral Australian/Papuan Y-pool on the Asian main land. *B. Horst[1], P. Forster[2], A. Eigel[3], B. Brinkmann[1]*. 1) Institut fuer Rechtsmedizin, Universitaet Muenster, Germany; 2) The McDonald Institute, University of Cambridge, United Kingdom; 3) Institut fuer Humangenetik, Universitaet Muenster, Germany.

Within recent years worldwide systematic genetic studies have been started to investigate the diversity of the human genome. Medical benefits could be obtained by the assessment of the prevalence and distribution of inherited deseases in the different populations of the world. Also understanding normal population variations is of great interest in regard to anthropological goals. Regarding migration of modern humans into Asia it is thought today that at least two independent migration waves took place. 50,000 to 70,000 years ago a southern wave brought people through Ethiopia along the southern coastal line to New Guinea and Australia. A second expansion started 50,000 years ago in nothern Africa to Central- and North-Asia, the Arctic and also America. Phylogenetic trees based on genetic distances have shown unstable clustering of Southeast Asian populations with New Guineans/Australians and other non-Africans. Short Tandem Repeat (STR) polymorphisms on the non-recombining part of the Y-chromosome are especially useful for the study of male specific lineage evolution because they are only influenced by mutation events and not by meiotic recombination. Because of the multitude of plausible trees it is often a challenging task to reconstruct phylogenies from human Y-chromosomal DNA variation. Networks are a new method to express a large number of trees by displaying alternative potential evolutionary paths. We constructed networks from population data of five Asian / Australian populations (Japanese, Han-Chinese, Thais, Papuans and Aboriginies) comparing two different network methods. All individuals were typed for the STR-loci DYS19, DXY156-Y, DYS389 (with its four subsegments), DYS390, DYS391, DYS392 and DYS393 on the Y-chromosome. In this context we were especially searching for traces of the ancestral Australian/Papuan Y-pool on the Asian mainland.

Program Nr: 1134 from the 1999 ASHG Annual Meeting

Secondary sex ratio in the offspring of Finnish retinoschisis carrier females. *L.L. Huopaniemi[1,2], J. Fellman[2], A. Rantala[1,2], A. Eriksson[2], H. Forsius[2], A. de la Chapelle[2,3], T. Alitalo[1,2,4].* 1) Dept of Medical Genetics, Haartman Institute, University of Helsinki, Helsinki, Finland; 2) The Folkhalsan Institute of Genetics, Departments of Molecular Genetics and Population Genetics,Helsinki, Finland; 3) Comprehensive Cancer Center, Ohio State University, Ohio, USA; 4) Depts. of Obstetrics and Gynecology, Helsinki University Central Hospital, Helsinki, Finland.

X-linked retinoschisis (RS) is the most common cause of juvenile macular degeneration. The disease causes progressive impairment of vision with variable degrees of severity. The gene defective in retinoschisis, XLRS1, was recently cloned and more than 90 disease causing mutations have been identified. XLRS1 is believed to be expressed solely in the retina. Three founder mutations contribute to the high incidence of RS in Finland. Already in the 1960s it was noticed that the Finnish RS carriers tend to have more sons than daughters. In our present study, we have analyzed the carrier status for a single widespread founder mutation (214G>A) of 202 females, and compared the secondary sex ratio (SSR) in the offspring of the 149 carrier females with reported normal SSR values. Our statistical analysis suggests that the SSR in the children of RS carrier females (SSR 129.8) is significantly higher than in the normal Finnish population (SSR 106). In order to evaluate factors which might contribute to the skewed secondary sex ratio, we performed a RT-PCR assay to check the expression of XLRS1 in various pregnancy and reproduction related tissues. We found that XLRS1 is expressed in the uterus, but not in the other tissues studied. We hypothesize that the 214G>A mutation leads to sex-specific or sex-limited changes in embryonic implantation or survival.

Program Nr: 1135 from the 1999 ASHG Annual Meeting

Are the familial determinants of lipid levels the same in diabetic and non-diabetic individuals? *G. Imperatore, W.C. Knowler, J. Roumain, R.L. Hanson.* Diabetes Arthritis Epidemiolog, NIDDK/NIH, Phoenix, AZ.

Linkage analysis of quantitative traits related to cardiovascular diseases, such as serum total cholesterol (TC), serum total triglyceride (TG), and high density lipoprotein cholesterol (HDL) concentrations, may help to identify susceptibility loci for these complex disorders. Diabetes is highly prevalent in some populations and it is possible that different loci may be involved in diabetic and non-diabetic individuals. Therefore, we estimated the correlation between the familial determinants in diabetic and non-diabetic siblings for these quantitative traits in 1232 Pima Indian sibships.

The sibships included 1734 diabetic and 2256 non-diabetic persons aged at least 20 years. Covariance components methods were used to estimate, for each trait, the heritability (including genetic and familial environmental effects) within diabetic siblings, heritability within non-diabetic siblings, and the covariance (reflecting the correlation) between diabetic and non-diabetic siblings. These parameters were estimated for TC, TG, and HDL. Prior to analysis each trait was log-transformed and adjusted for age and sex, and, among diabetic individuals, for duration of diabetes.

For TC heritability was 48% within non-diabetic siblings and 31% in diabetic siblings. For TG heritability was 44% in non-diabetic siblings and 42% in diabetic siblings. For HDL heritability was 33% in non-diabetic siblings and 34% diabetic siblings. These traits were also significantly ($P < 0.0001$) correlated among siblings discordant for diabetes (heritabilities = 37%, 47%, and 45% for TC, TG, and HDL). These estimates imply that correlations between familial determinants in diabetic and non-diabetic siblings are > 0.96 for each trait.

Familial determinants of TC, TG, and HDL are largely the same in diabetic and non-diabetic individuals. Linkage analyses of these traits are justified in pooling diabetic and non-diabetic persons, after adjusting for diabetes.

Program Nr: 1136 from the 1999 ASHG Annual Meeting

Chinese surnames are polyphyletic in origin: Evidences based on 19 Y-SNPs. *L. Jin[1,2], B. Su[2], J. Xiao[1], J. Akey[2], D. Lu[1], D. Shen[2], R. Chakraborty[2], J. Tan[1]*. 1) Institute of Genetics, Fudan University, Shanghai, China; 2) Human Genetics Center, University of Texas - Houston, TX.

Analysis of surnames for genetic purposes has been suggested and widely used due to their nature of patrilineal transmission in most of the populations. Surnames appeared in China at least 3000 years earlier than in Europe or Japan and there are between three and four thousand surnames used by the Han Chinese. A genetic analysis of a general Han population of 1,500 male individuals born in 28 different Provinces across China has been conducted at 19 Y chromosome single nucleotide polymorphic markers (Y-SNPs). The results of this analysis demonstrate that large isonymous populations tend to have multiple distinct haplotypes, implying a polyphyletic origin. This observation is consistent with the historical records that in general, the larger an isonymous population is, the more origins that surname has. Therefore, it is not necessarily a valid approach to use the number of surnames to estimate the number of founders in a Chinese population (either isolated or panmictic) without a genetic study of Y chromosome markers.

Program Nr: 1137 from the 1999 ASHG Annual Meeting

The survival of persecuted Jews in Spain and Portugal in the 15th century led to endogamic preservation and emergence of an autosomal recessive retinitis pigmentosa mapped to chromosome 15q22. *J. Kaplan[1], S. Gerber[1], J.-M. Rozet[1], L. Santos[2], L. Lopes[2], O. Gribouval[1], I. Perrault[1], D. Ducroq[1], F. Ferraz[3], A. Munnich[1].* 1) Unité de Recherches sur les Handicaps Génétiques de l'Enfant, INSERM U393, Hôpital Necker, Paris, France; 2) Servico de Ophtalmologia, Hospital Dos Capuchos, Lisboa, Portugal; 3) Servico de Genetica, Hospital Dona Estefania, Lisboa, Portugal.

The last Marranos are the survivors of Spanish Jews persecuted in the late fifteenth century, escaped in Portugal and forced to convert to save their lives. Isolated groups still exist in mountainous areas such as Belmonte in the Beira Baixa province of Portugal. We report here the genetic study of a highly consanguineous endogamic population of Crypto Jews of Belmonte affected with autosomal recessive retinitis pigmentosa (RP). A large-scale linkage analysis was carried out in order to localize the disease-causing gene. All previously known autosomal recessive RP loci were first excluded in this population. Then, an exclusion map was performed using fluorescent oligonucleotides of the Genescan Linkage Mapping Set, version II (Perkin-Elmer) at an average distance of 10 cM throughout the genome. A total of 239 polymorphic markers were studied in this population and six markers were constantly found homozygous in nine affected individuals providing evidence of close proximity of these markers with respect of the disease locus on chromosome 15q22. No known RP gene has been mapped to this interval, but the gene encoding the retinal rod Na-Ca-K exchanger (SLC24A1) and mapping to the 15q22 region, was excluded. Subsequently, starting from flanking markers, a YAC contig covering the genetic interval was constructed. Interestingly, a gene which expression is highly restricted to the outer nuclear layer of retinal photoreceptor cells was found to map to one YAC of this contig. A search for mutations was undertaken allowing to ascribe the RP in this population to an homozygous mutation in this gene. Preliminary haplotype studies support the view that this mutation is relatively ancient and will provide help tracing the origin and migration of Sephardi Jews.

Program Nr: 1138 from the 1999 ASHG Annual Meeting

Mitochondrial DNA Haplotype Studies in Colombian Amerindian Populations show two Possible Migration Routes into South America. *G. Keyeux[1], C. Rodas[1], D. Carter[2], J.E. Bernal[1]*. 1) Inst de Genetica Humana, Univ Javeriana, Bogota, DC, Colombia; 2) Microbiology Dept, Univ of Sydney, Australia.

Mitochondrial DNA haplotype studies as well as D-loop sequencing have been useful in studying the origins of Native American populations. Four main haplotypes and possibly a fifth lineage are defined by a few restriction sites and deletion/insertion polymorphisms and a limited set of control region mutations. Haplotypes have been characterized in North-, Central- and South-American Amerindian, North American Nadene and Aleutian populations, and also Siberian, Central-Asian and South-East Asian populations in the hope of fully depict the route(s) of migration between Asia and America in the past 25.000 years. Very little of the South American settlement is known and Colombia, presumably an obligate turning-plate in the migrations between North- and South America, had not been studied yet. In an effort to remedy this, we have analyzed mitochondrial haplotypes in 25 different ethnic groups from all over Colombia. Our results indicate a varied distribution of the four founder A-D lineages in the different populations. Some private polymorphisms, and the presence of other lineages, some presumably corresponding to other Asian haplotypes, were also detected in a few populations. The distribution of A/D haplotype frequencies in our groups, which is inverted once the natural barrier of the Andes cordillera is reached, points to possibly two migrational waves into South America. These two migrational waves relate the Central American and North-west Colombian groups, on the one side of the Andes, and the Lowland-Amazonian and South American groups on the other side.

Program Nr: 1139 from the 1999 ASHG Annual Meeting

Assessment of Medical Insurance Benefit Data on Congenital Anomalies in Korea. *S-S. Kim[1], S-C. Jung[1], K-S. Yoon[2], H-J. Kim[3], M-J. Kwon[4], J-S. Lee[1,5]*. 1) Div. of Genetic Diseases, National Institute of Health, Korea; 2) Eulji Medical Coll., Korea; 3) Ajou Univ. Coll. of Medicine, Korea; 4) Sungnam Inha Hosp., Inha Univ., Korea; 5) Yonsei Univ. Coll. of Medicine, Korea.

To access the accuracy of Medical Insurance Treatment Data(MITD) as a source to estimate prevalence of congenital anomalies, the correspondence of ICD 10 disease codes in MITD was evaluated through case by case comparison of medical records from 3 university hospitals(n=3,528). Medical Insurance encompassed 97.9% of total Korean population in 1996 and 53,102 children under 4 years old took medical examination with complaints of congenital anomalies. The correspondences of MITD with medical records were 92.1% for in-patients and 56.6% for out-patients with ICD 10 three character disease categories. Medical records for some patients could not be reviewed because there were no corresponding records with identical personal identification codes(6.0% for in-patients, 10.2% for out-patients). It was found through reviewing medical records that a cardiovascular malformation accompanied with other defects in the same category, and just one principal diagnosis was reported in MITD. Disease codes for cleft lip and/or cleft palate were described without strict distinction in medical records. So, after categorize three character disease codes included in congenital cardiovascular anomalies or cleft anomalies respectively, the overall agreement rise to 97.1% for in-patients and 81.7% for out-patients in infant under 1 year old. There were differences in correspondence among congenital anomalies by system ranging from 79.3% to 99.3% for in-patients and from 33.3% to 85.0% for out-patients. The estimated birth prevalence of several congenital anomalies considering above agreement were 9.1 per 1000 infants for congenital anomalies of circulatory system(Q20-Q28), 3.4 for other congenital anomalies of the digestive system(Q38-Q45), and 3.8 for congenital anomalies of eye, ear, face and neck(Q10-Q18). Our study showed that MITD can give limited but useful information on the outline of congenital anomalies in Korea where National Surveillance System or Registry for congenital anomalies does not yet established.

Program Nr: 1140 from the 1999 ASHG Annual Meeting

Prospective epidemilogic study of birth defects in Korea, a community based pilot study. *Y.K Kim[1], H.J. Kim[2]*. 1) Ajou Univ. School of Medicine, Suwon, Korea; 2) Genetics Clinic & Lab. of Medical Genetics, Ajou Univ. School of Medicine, Suwon, Korea.

This prospective epidemiological study of birth defects is a part of national effort to characterize congenital birth defects & genetic disorders in Korea and to establish National Genetic Database. The registry form was designed to identify, Down syndrome, facial cleft (cleft lip/or cleft palates), ONTD, polydactyly, ambiguous genitalia & other major malformations. The study was conducted in all hospitals in the province of Suwon, total 34 hospitals delivering one to 300 pregnancies per month, from May 1, 1997 to April 30, 1999 for two years. All outcome of the pregnancies including liveborns, stillborns and TOPs (20weks gestation) were subject of the evaluation for the presence of above major birth defects. Each delivery unit was requested to fill out registry form and monthly statistics were collected and analyzed. In addition, medical records of two tertiary hospitals on babies born during the study period were also searched and analyzed. The total number of delivery was 30,319 including 332 twins and 1 triple gestations, yielding 30,653 babies including 270 stillbirth and 26 TOPs (>20weeks gestation). The overall incidence of congenital anomalies was 1:86 (357 cases among 30,653 babies). Down syndrome was reported in 14 liveborns and 11 TOPs, an incidence of 1:125, facial clefts were 48 liveborns, 6 stillbirth, and 1 TOP, an incidence of 1: 557, ONTD was 6 liveborns and 2 TOPs, an incidence of 1:3,832, polydactyly was 15 liveborns, an incidence of 1;2,021, and ambiguous genitalia was 3 liveborns. The incidences of 1:557 in facial clefts was higher and 1:3,832 in ONTD was lower than any reported ethnic data. Search for neonatal and pediatric medical record for birth defects yielded 39% of total anomalies indicating the importance in generating accurate data. This pilot study represented the first community based data on birth defects in Korea, differing from most of previous studies based on single tertiary hospital and the first comprehensive birth defects data ascertained from not only liveborns but also stillbirth including TOPs following abnormal prenatal diagnosis.

Program Nr: 1141 from the 1999 ASHG Annual Meeting

Microsatellite-based statistics for inferring the past population growth. *J.P. King[1], M. Kimmel[1], R. Chakraborty[2].* 1) Dept Statistics, Rice Univ, Houston, TX; 2) Human Genetics Ctr, Univ Texas, Houston, TX.

Recently, several statistics were developed for detection of past population growth, based on short tandem repeat (STR) loci, also referred to as microsatellite loci. Some of their properties were investigated in the original communications, although never were they systematically compared to each other. Microsatellite loci are an important source of information about the evolution of human genome. Also, they were found of crucial importance in gene mapping and estimation of individuals' relatedness and identity. We present results concerning the power to detect past population growth using three microsatellite-based statistics available in the current literature: (1) based on between-locus variability, (2) based on the shape of allele-size distribution, and (3) based on the imbalance between variance and heterozygosity at a locus.

The analysis is based on the single-step stepwise mutation model (SSMM). The main feature evaluated is the power of the statistics under variable mutation rate. This is an important point, since it is a standard procedure to pool data collected at a number of loci and mutation rates at these loci may not be the same. Other characteristics, such as bias and variance of the indices relative to their expectations, also are considered. Our analysis indicates that the statistic based on the imbalance between variance and heterozygosity at a locus has the highest power for detection of population growth, in particular when mutation rates vary across loci. (Research supported by NIH grants GM 41399, GM 45861, and by Keck's Center for Computational Biology at Rice Univ.).

Program Nr: 1142 from the 1999 ASHG Annual Meeting

Natural variation of the androgen receptor gene CAG repeat in human populations. *R.A. Kittles[1,2], D. Young[1,2], D. Parish-Gause[1,2], G.M. Dunston[1,2].* 1) National Human Genome Center at Howard University; 2) Department of Microbiology, College of Medicine, Howard University, Washington, DC.

The androgen receptor (AR) interacts with androgens to promote cell division in the prostate gland. Exon 1 of the AR gene contains a polymorphic CAG repeat sequence whose length is inversely related to AR transcriptional activity. Increased prostate cancer risk, particularly among African Americans has been attributed to CAG repeat lengths less than 20 repeats. However little is known about the normal range of variation for the CAG locus. Here we examine variation at the AR CAG repeat locus in 498 unrelated healthy African American males between 55 and 70 years of age. West Africans (Liberians n=33; Ghanaians and Nigerians n=42), Euroamericans (n=97), and Amerindians (n=81) were included for comparative purposes. Alleles at the AR CAG locus were typed using fluorescent dye detection using an ABI 377 DNA sequencer following PCR amplification.

Twenty-seven alleles ranging from 5 to 31 repeats were observed in the populations. Not surprisingly, African Americans possessed the largest number of alleles (k=22) and highest gene diversity (0.926 ± 0.05). Mean repeat length varied considerably between populations from 20.6 ± 2.9 to 16.4 ± 2.9 in Amerindians and Liberians, respectively ($P=7 \times 10^{-8}$). Populations of African descent possessed smaller CAG repeat lengths than the Amerindian and Euroamerican populations ($P<0.00001$). F_{ST} analyses revealed that the sample of African Americans was more closely related to Liberians than to Ghanaians and Nigerians. Significant genetic differentiation was observed between African Americans and the two non-African populations ($P<0.000001$). These results reveal that variation in androgen receptor CAG repeat length differs considerably between human populations. In our sample of healthy African American men, 70% contained CAG alleles shorter than 20 repeats while the Amerindian population only contained 30% of the shorter repeats. Our results, along with other genetic, nutritional and socioeconomic factors, may be important in understanding the increased risk of prostate cancer in African Americans.

Program Nr: 1143 from the 1999 ASHG Annual Meeting

Methylenetetrahydrofolate reductase genotypes and predisposition to atherothrombotic disease: evidence that the wild type '677 CC' genotype is protective. *L.A.J. Kluijtmans, A.S. Whitehead.* Pharmacology, University of Pennsylvania, Philadelphia, PA.

Hyperhomocysteinemia is an independent risk factor for atherothrombotic disease. Recent evidence indicates that individuals homozygous for the methylenetetrahydrofolate reductase (MTHFR) 677C allele exclusively accumulate 5-methyltetrahydrofolate, the methyl donor for homocysteine conversion, in their red blood cells, in contrast to 677 TT homozygotes who also accumulate significant levels of non-methylated folate derivatives. The latter suggests that the MTHFR 677 TT, CT and CC genotypes are qualitatively different with respect to folate utilization and hence capacity to remethylate homocysteine, and consequently raises the question whether all three genotypes confer different levels of atherothrombotic risk. A "restricted" meta-analysis of subjects from the first ten studies reporting a significantly increased risk of atherothrombotic disease conferred by the TT genotype was performed. After proportional adjustment for the greater number of TT homozygotes, the CC and CT frequencies observed in cases were compared to expectations based on the frequencies of these genotypes in controls. The observed CC frequency among cases was lower than expected in 8 of the 10 studies. In the meta-analysis, which included 1,857 cases and 2,942 controls, 643 (34.6%) cases, instead of the 713 (38.4%) expected, had the MTHFR CC genotype (P=0.010). Our findings suggest that the three MTHFR C677T genotypes confer different levels of atherothrombotic risk, and therefore strengthen the hypothesis that the MTHFR TT genotype is an important determinant in 'at risk' populations. Furthermore, the significantly decreased CC homozygote frequency among cases relative to expectation implies that this genotype is protective against atherothrombotic disease. This observation, for which a biologically plausible mechanism is proposed, is of considerable public health importance.

Program Nr: 1144 from the 1999 ASHG Annual Meeting

Effect of high consanguinity rate on the incidence of phenylketonuria in Iran. *J. Koochmeshgi, S.M. Hosseini-Mazinani, P. Nader-Esfahani.* Molecular Genetics, National Research Center for Genetic Engineering and Biotechnology, Tehran, Iran.

Phenylketonuria (PKU), the most common inherited error of amino acid metabolism, is an autosomal recessive disease caused by mutations in the phenylalanine hydroxylase (PAH) gene. The incidence of PKU varies widely among different populations. The highest incidence of PKU to date has been reported from Iran's neighboring country, Turkey. Accumulating evidence indicate a high incidence for this disorder in Iran. Researchers studying the most prevalent mutation in the PAH gene causing phenylketonuria in Mediterranean populations, the IVS10nt546 mutation, have based their differing hypotheses about the geographical origin and pattern of diffusion of this mutation partly on their differing views on the relative importance of the rate of consanguinity and the frequency of the PAH gene mutations in the countries of this region - e.g. Cali, F. et al. Human Genetics 100,350-355(1997). To investigate the role of parental consanguinity in the high incidence of PKU in Iran, we studied 109 unrelated Iranian PKU patients. For 83 patients (76%), the parents were consanguineous. Our data suggest a very important role for consanguinity in the high incidence of PKU in Iran.

Program Nr: 1145 from the 1999 ASHG Annual Meeting

Frequency estimation for rare Y-chromosomal haplotypes. *M. Krawczak[1], L. Roewer[2].* 1) Medical Genetics, University of Wales, Cardiff, UK; 2) Institut für Gerichtliche Medizin, Humboldtuniversität, Berlin, Germany.

Microsatellite markers located on the Y chromosome have enormous potential for use in forensic medicine and kinship testing. However, since most of the human Y chromosome is non-recombining, quantification of the statistical evidence provided by a positive match is difficult. Multiplication of allele frequencies to estimate haplotype frequencies is not justified and, as emerges from empirical data, can sometimes be misleading. We have therefore developed a numerical method to obtain Y-chromosomal haplotype frequency estimates via extrapolation, based upon patterns of association observed between subhaplotypes. Application to data from 1819 Caucasian males typed for markers DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393 and DYS385 reveals that this methodology is highly accurate for haplotypes that are already included in the database. For previously unobserved haplotypes, however, prior inclusion for the purpose of frequency estimation appears overly conservative. We suggest a compromise by which a new haplotype is used for the study of subhaplotype association but is given no weight in the actual extrapolation step. This approach ensures that rare haplotypes retain their high evidential power even if the database used for estimation is of realistic, and therefore moderate, size.

Program Nr: 1146 from the 1999 ASHG Annual Meeting

Causes of spouse blood pressure correlation in a Northeastern Brazilian sample. *H. Krieger[1,2], M. Province[1], D.C. Rao[1]*. 1) Division of Biostatistics, Washington University School of Medicine, St.Louis, MO; 2) Dept Parasitologia, Universidade de Sao Paulo, Sao Paulo, Brazil.

A large sample (1801 nuclear families) from a migrant Northeastern Brazilian population was surveyed with the scope of uncovering the causal relationships among Systolic (SBP) and diastolic (DBP) blood pressures, ethnicity (E) and Body mass index (BMI). All the variables showed significant spouse correlations overall (0.41, 0.12, 0.08, 0.12, for E, BMI, SBP and DBP, respectively). A tetra-variate path analysis model was applied to the data to estimate the impact of cohabitation time and ethnicity on the correlation structure. Neither BMI nor the BP spouse correlations varied significantly with cohabitation time, while the spouse correlation of ethnicities, as measured by the proportion of black admixture, is significantly associated with cohabitation time, indicating that a conspicuous breakdown of isolates has taken place in this population, during the 60s decade. The proportion of the BP correlations attributable to BMI correlation, although significant, is less than 2% of the expected correlation among spouse's BPs. On the other hand, the Ethnicity contribution is rather high (around 10% for the average cohabitation time), suggesting that in populations with rather high levels of ethnical admixture, ethnical homogamy is one of the most important causes of positive blood pressure correlation among spouses.

Program Nr: 1147 from the 1999 ASHG Annual Meeting

Validation of pedigree data in the presence of genotyping error. *J. Kumm, S. Browning, E.A. Thompson.* Statistics, University of Washington, Seattle, WA.

Analyses of genome scan data typically assume marker genotypes to be observed without error. This is undesirable, both in the validation of pedigree relationships and in linkage detection. We have developed a program, based on the Baum algorithm, to compute likelihoods at multiple linked loci, in the absence of genetic interference, allowing for typing error. Using real data on a 488-marker scan on sib pairs, we found that on the basis only of Chromosome 1 data, sibs (S) are often not well distinguished from halfsibs (H), but that the LOD (base 10) relative to unrelated (U) is usually at least 3. With data on Chromosomes 1-5, S is normally well distinguished from H, and U is rejected (LOD 15 to 20), and with a full genome scan the LOD for S vs H is typically at least 15. Quantiles of the full-genome LOD distribution suggest a median LOD of truth vs U of 152 for sibs and 42.4 for halfsibs.

More general analyses have used real and simulated data at 100 markers on Chromosomes 1-5, using a model of independent errors at rate e. A modification excluding the possiblity of two errors in the same one-marker genotype significantly increases computational efficiency, permitting a full genome analysis including markers with more than 20 alleles. Likelihoods for putative sibs, halfsibs and unrelated pairs are computed for each of these same three relationship hypotheses. Typically, with real data the MLE $\hat{e}$ is 3 to 5%, whereas for the simulated data $\hat{e}$ is 0. For e up to 5%, relationship lod scores seldom change by more than 3. However, the algorithm also provides the posterior probability of marker genotypes, given the complete genome scans on the pair of relatives, and hence is a powerful method of detecting errors and localizing recombination events. Moreover, allowance for error is critical when a true relationship may be otherwise excluded, such as for paternity or in the joint analysis of sib trios, where a third sib can provide powerful validation (or refutation) of a putative sib pair.

Work supported by NIH grant GM-46255.

Program Nr: 1148 from the 1999 ASHG Annual Meeting

Archaic lineages in modern human populations. *D. Labuda, E. Zietkiewicz, V. Yotova.* Sainte-Justine Hospital, Research Center, Department of Pediatrics/University of Montreal, Montreal (Quebec) H3T 1C5 Canada.

Excess in protein, mitochondrial and nuclear DNA diversity among Sub-Saharan Africans was either taken as evidence of greater antiquity of African populations or explained by a relatively larger effective population size of Africans in the recent past. Among 36 segregating sites previously found in dys44, an 8 kb DNA segment from the dystrophin gene, 21 were distributed worldwide, 13 were specific to sub-Saharan Africans and only two were absent from the African sample. The age distribution of these polymorphisms, based on their new allele frequencies, indicated that the specific variants originated less than 150,000-50,000 years ago, suggesting that the excess of African diversity accumulated after the divergence.

The present study concerns dys44 haplotypes analysed in a worldwide sample of 868 X-chromosomes. In contrast to expectations, African-specific polymorphisms occur clustered on the same background haplotypes and show decayed linkage disequilibrium. These and other characteristics indicate that African-specific sites have a longer genetic history than that suggested by prior age estimates and must have originated before their appearance in the present-day populations. Our findings can be explained by genetic hybridization of the ancestral humans expanding outside and into Africa with local populations from more archaic, albeit related lineages. *Supported by the Medical Research Council of Canada..*

Program Nr: 1149 from the 1999 ASHG Annual Meeting

What predisposes long normal GAA allele to full expansion in Friedreich ataxia? GAA variability and haplotype studies in different human groups. *M.Z. Labuda[1], N.E. Barucha[2], M. Pandolfo[1]*. 1) Ctr de Recherche LC Simard, CHUM, Montreal, PQ, Canada; 2) Bombay Hospital, Mumbai, India.

Friedreich ataxia (FRDA) is an autosomal recessive neurodegenerative disorder with a carrier frequency of about 1:90 in Caucasians. Over 95% of the FRDA causing mutations are GAA-repeat expansions in the first intron of the frataxin gene. All chromosomes with expansion share the same four-marker haplotype (FAD1 ITR4, ITR3, CS2) or can be derived from the consensus A-T-C-C haplotype by 1-2 recombinations. Interestingly, chromosomes carrying long normal (LN) alleles (16-34 GAA) also carry the same haplotype. This support the concept that they form a reservoir for recurrent expansions, independently confirmed by direct observation of new expansions. Recently we tested FRDA patients from India and Ecuador, and found that they all carry expansion associated with the European A-T-C-C haplotype suggesting a single origin of all expanded alleles in Indo-Europeans. Among FRDA patients sent to us from multi-ethnic Centers in Canada and USA, 170 carried GAA expansion(s). Of these, all were of Indo-European or Middle East descent. To our knowledge no patient of non-European origin with GAA expansion in frataxin gene was reported. In order to get an insight into the origin of expansion mutation we tested the GAA polymorphism in different human groups; Amerindians (n=30) mixed Orientals (n=47), African Americans (AA) (n=43). We identified two Orientals and fourteen (32%) AA to carry LN alleles (12-23 repeats). All but one of these alleles carried pure GAA, similar to European LN chromosomes. The high frequency of LN alleles among AA can not be accounted for by admixture with Caucasian chromosomes where only 18% of LN alleles are observed. Since LN alleles in AA do not seem to be prone to expansion we are presently investigating the ancestral relation between LN chromosomes of AA and European origin. In summary, our results further support the notion of a single origin of GAA expanded chromosomes in the human history and suggest that in addition to the presence of LN allele there are other features of A-T-C-C chromosome that predispose to full expansion.

Program Nr: 1150 from the 1999 ASHG Annual Meeting

Genome-wide linkage analysis of human fatness: The HyperGEN Blood Pressure Study. *C.E. Lewis[1], D. Arnett[2], I. Borecki[3], H. Coon[4], R.C. Ellison[5], S. Hunt[4], A. Oberman[1], S. Rich[6], M.A. Province[3]*. 1) Preventive Medicine, Univ. of Alabama-Birmingham, Birmingham, AL; 2) Epidemiology, Univ. of Minnesota, Minneapolis, MN; 3) Biostatistics, Washington Univ., St. Louis, MO; 4) Cardiovascular Genetics, Univ. of Utah, Salt Lake City, UT; 5) Preventive Medicine & Epidemiology, Boston Univ., Boston, MA; 6) Bowman Gray, Winston-Salem, NC.

Measures of human adiposity and body composition exhibit substantial familial resemblance; however, the mode of inheritance is complex. We conducted a full genome search for genes potentially influencing two related phenotypes: body mass index (BMI, kg/m^2) and percent body fat (PBF) from bioelectric impedance. Genotypes for 387 markers spaced roughly equally throughout the genome (CHLC-8) were typed by the NHLBI Mammalian Genotyping Service (Marshfield, WI) on the first 579 Caucasian hypertensive sibpairs and 247 African-American hypertensive sibpairs recruited by the HyperGEN network, a study of hypertension genetics in the NHLBI Family Blood Pressure Program. Hypertension was defined as BP³140/90 or on anti-hypertensive Rx. The search for quantitative trait loci for obesity phenotypes was carried out using the “model-free” variance component method as implemented in SEGPATH (Province et al. 1999). Each of the phenotypes was adjusted for the effects of age, within sex and race, and the linkage analysis was carried out separately within race using group-specific marker allele frequencies from additional random samples. The highest lod score detected was 2.5 for PBF near the telomere of 1p in the Caucasian sibs. The lod score for BMI in Caucasians was 2.3 at this same location, providing some confirmatory evidence. However, lod scores for both phenotypes were nearly zero in the African-American sibs at that location. Suggestive linkages were also found for BMI on chromosome 3p (lod=2.2) and for PBF on 10q (lod=2.1)and 12q (lod=2.1)in the Caucasians, while the highest lod score in the African-American sibs for these phenotypes was only 1.6 (chromosome 7). The current analysis represents only about the first half of the sibships collected in HyperGEN; a replication phase is underway, along with finer mapping in the most promising regions.

Program Nr: 1151 from the 1999 ASHG Annual Meeting

Molecular epidemiology analysis of human germline mutation in Mainland China. *X. LI[1], J-z. Liu[2], J. Drost[1], E.C. Thorland[3], Q. Liu[1], T. Lind[3], S. Roberts[3], H.Y. Wang[2], S.S. Sommer[1].* 1) Molecular Genetics, City of Hope/Beckman Research, Duarte, CA; 2) Inst. Medical Sciences, Chinese Acad.Med.Sci., Beijing, China; 3) Mayo Clinic/Foundation, Rochester, MN.

Germline mutations are the major source of genetic variation that allow a species to evolve over time but at a cost of Mendelian disease and genetic predisposition to multifactorial diseases. Previous analyses have revealed a similar pattern of germline mutations in the human factor IX gene (*F9*) among a variety of ethnically and geographically diverse populations that is compatible with the ancient pattern that has shaped the mammalian genome, primarily by germline mutations from endogenous mechanisms. Here, we compare the pattern of germline mutation in a population of hemophilia B patients from Mainland China (n=66), which constitutes one-fifth of the world's population, to that in U.S. Caucasians (n=213), Blacks (n=34), and Mexican Hispanics (n=31) (p=0.8 and 0.9, six and eleven mutations categories, respectively in a global analysis). The similar pattern of germline mutation in all ethnic groups studied to date is compatible with the hypothesis that endogenous processes predominate in human germline mutations.

Program Nr: 1152 from the 1999 ASHG Annual Meeting

DNA polymorphism in the study of gene geography of East Europe. *S.A. Limborska[1], P.A. Slominsky[1], E.V. Balanovskaya[2], O.V. Belyaeva[1], T.V. Pogoda[1], M.I. Shadrina[1], S.N. Popova[1], O.P. Balanovsky[1], D.A. Verbenko[1], E.K. Khusnutdinova[4], A.I. Mikulich[5], L.V. Bets[3], A.V. Stepanova[3], V.A. Spitsyn[2].* 1) Inst of Molecular Genetics, RAS, Moscow, Russia; 2) Medical Genetic Scientific Center, RAMS, Moscow, Russia; 3) Moscow State University, Moscow, Russia; 4) Bashkir Science Center of Ural Branch of RAS, Ufa, Russia; 5) Institute of Ethnography, Minsk, Byelarus.

We have analysed the normal variability of mini- and microsatellite loci and also insertion/deletion polymorphism in 32 East European populations with different level of Caucasoid and Mongoloid components. Different types of synthetic maps were constructed using reliability theory. Four maps od genetic distances were prepared based on calculation of distances from mean gene frequencies of these linguistic families (Indo-European, Uralic and Altaic) and from total mean frequencies of East European region at all. The data reveal the influence of gene pool of each people on the neighboring populations, that provides the interaction of various ethnic factors in the forming of the gene pool of this area. At the same time the results show that each ethnic-linguistic community keeps its own geneic characteristics distinguished from others.

Program Nr: 1153 from the 1999 ASHG Annual Meeting

Haplotype analysis at the FRAXA locus in Thai subjects showing no founder effect. *P. Limprasert[1], V. Saechan[1], N. Ruangdaraganon[2], T. Sura[2], P. Vasiknanonte[1], W.T. Brown[3]*. 1) Faculty of Medicine, Prince of Songkla Univ, Songkhla, Thailand; 2) Ramathibodi Hospital, Mahidol Univ, Bangkok, Thailand; 3) New York Institute for Basic Research, Staten Island, NY, USA.

The prevalence of fragile X syndrome (FXS) is approximately 7 % in developmentally delayed Thai boys. This is somewhat high compared to previous studies. To determine if FXS may have a specific haplotype association we analyzed 91 unrelated control subjects and 19 unrelated FXS patients using 3 markers (DXS548, FRAXAC1 and FRAXE) and a single nucleotide polymorphism (ATL1: A or G). FRAXAC1 and DXS548 are located ~ 7 Kb and ~ 150 Kb proximal to the FRAXA-CGG whereas ATL1 and FRAXE are located ~ 5.6 Kb and ~ 600 Kb distal to the FRAXA-CGG. We found 36 haplotypes in the contol group and 14 haplotypes in the FXS group. No significant association of a specific haplotype in either the control or FXS groups was found. Interestingly, of 14 haplotypes in the FXS group, 7 haplotypes were not found in the control group possibly suggesting new mutations or admixture of immigrant haplotypes. We observed that most diverse haplotypes came from different FRAXE alleles. This may reflect that recombination or mutation involving the FRAXE was involved. For this reason we analyzed haplotypes from the remaining markers (DXS548-FRAXAC1-ATL1). We found 2 major haplotypes (20-18-G and 20-19-A) with no significant haplotype differences between the control group (60/91 of 20-18-G and 20/91 of 20-19-A) and FXS group (11/19 of 20-18-G and 5/19 of 20-19-A). The other haplotypes were found 11/91 in the control group and 3/19 in the FXS group. This suggests that no founder chromosome is associated with Thai FXS. These findings contrast with most other reports on FXS founder effects in different ethnic groups. Our data imply that the Thai FXS chromosomes may originate independently in unrelated individuals.

Program Nr: 1154 from the 1999 ASHG Annual Meeting

Association study of aldose reductase gene polymorphism and diabetic nephropathy in Chinese population. *J. Liu, L. Ji, X. Han.* Department of Endocrinology and Metabolism, People's Hospital, Beijing Meidcal University, Beijing, P.R.China.

Diabetic nephropathy (DN) is one of the major causes of morbidity and mortality in patients with diabetes mellitus. Recently studies have shown that genetics play a important role in the predisposition to DN. Aldose reductase (AR) play a important role in the pathogenesis of DN and, clinically , AR inhibitor is effective in controlling the progression of DN. Several studies have suggested that polymorphism of AR gene is associated with susceptibility of DN and DR. Goal: To test the hypothesis that AR gene contributes to the genetic predisposition to DN in Chinese NIDDM population. Study design: A case--control studies was carried out to investigate the allelic distribution of AR gene polymorphism in the following groups of individuals: 1). Normal subjects of the Han Nationality living in northern China (n=117), 2) NIDDM control: long-standing non-insulin-dependent-diabetes mellitus(NIDDM) patients without DN and diabetic retinopathy (DR) (n=76), 3) DN group : NIDDM patients with DN an DR (n=62) , and(4) DR group: NIDDM patients with DR but not DN (n=39). Results: (1) 9 alleles and 27 genotypes were identified in the subjects studied. (2) The frequency of Z+2 allele was significantly increased in DN group (DN vs. NIDDM control and DR group , P=0.041; DN group vs. DR group , P=0.037) Conclusions: Our results suggested that AR gene or gene nearby contribute to genetic susceptibility of diabetes nephropathy in Han Chinese living in northern China and diabetic patients carrying Z + 2 allele had a increased risk of developing DN.

Program Nr: 1155 from the 1999 ASHG Annual Meeting

Frequency of Common Thrombophilic Mutations in the South Carolina Population: Factor V Leiden, Prothrombin 20210, and MTHFR. *J.W. Longshore, K. MacClenahan, K. Brenkusova.* Molecular Diagnostic Lab, Greenwood Genetic Ctr, Greenwood, SC.

In the past few years, many of the molecular mechanisms involved with thrombosis have been identified. Among them are the factor V Leiden and prothrombin 20210A mutations which have been implicated in up to 65% of cases of inherited thrombophilia. Additionally, the MTHFR 677T mutation has been associated with an increased thrombotic risk and elevated plasma homocysteine levels. These mutations have been noted to occur frequently in the Caucasian population. In order to determine the frequency of these three mutations in the South Carolina population, a series of 512 consecutive births was studied. The population consisted of 254 males and 258 females with an ethnic distribution of 51.9% Caucasian, 43.9% African-American, 2.7% Hispanic, and 1.4% of other races. For factor V Leiden, 4.10% of the study population was heterozygous and a single homozygote was detected. Heterozygosity for the prothrombin 20210A mutation was detected in 0.78% of the consecutive births. As expected, the MTHFR mutation had the highest frequency with 35.9% of the study population being heterozygous and 7% being homozygous for the mutation. When the data was stratified for race, the Caucasian population had heterozygote frequencies of 5.64%, 1.5%, and 47% for factor V Leiden, prothrombin 20210A, and MTHFR 677T while the African-American population frequencies were 2.23%, 0%, and 22.32% respectively. Interestingly, coinheritance of these thrombophilic mutations was often noted in our screened population.

Program Nr: 1156 from the 1999 ASHG Annual Meeting

Methylenetetrahydrofolate reductase C677T and A1298C gene mutations in an inbred group of *Mazateco* Indians:An ethnic genetic marker for some Amerindian populations? *M.A. Lopez, J. Zuniga, J. Granados, O.M. Mutchinick.* Dept of Genetics[1], Immunology[2],Inst Nac Nutricion S Zubiran ,Mexico,DF.

MTHFR gene mutations have a worldwide distribution.The C677T transition has been extensively studied in many populations of similar and different ethnic groups.Prevalence of the homozygote TT varies from as low as 1.5% in blacks to as high as 34.8% in Mexicans (Mutchinick et al., 48th ASHG Meeting).More recently (1998) a new common mutation (A1298C) was described in Caucasians.While homozygous TT has a thermolabile enzyme that alter folate metabolism and induce a moderate hyperhomocysteinemia (HCY), the CC homozygote has a milder defect without HCY. Compound heterozygous behaves as TT homozygous. These genotypic arrays are recognized as important risk factors for neural tube defects (NTD) and cardiovascular disease (CVD). The high frequency of the C677T mutation observed in our population prompted us to study both mutations in 102 healthy Mazateco Indians. The aim was to know more about the prevalence of these MTHFR gene mutations and the potential disease risk they could represent for Amerindian populations. Blood samples were processed for DNA, MHC and other genetic markers. PCR amplified fragments of 142bp and 163bp were digested with TaqI and MboII restriction enzymes, followed by electrophoresis in agarose 4% and polyacrilamide 20%, to identify mutations C677T and A1298C respectively. Our results shows: 1) extremely high frequency of the C677T mutation with 70.6% of homozygous TT, and gene frequencies of 20.1 and 79.9% for C and T alleles respectively; 2) very low frequency of the A1298C mutation with 85.3% homozygous AA, and 0% homozygous CC, and gene frequencies of 92.6 and 7.4% for A and C alleles respectively, 3) very low prevalence (3.9%) of compound CT/AC heterozygotes, and 4) genotypes for C677T mutation are in H-W disequilibrium (p: <.001). MHC typing showed a high prevalence of an autochthonous otherwise rare haplotype. These results may suggest that the MTHFR C677T could be a very popular mutation in Amerindian populations, explaining in part the high prevalence of NTD in Mexico and some Central American countries, and representing maybe a higher risk for CVD.

Program Nr: 1157 from the 1999 ASHG Annual Meeting

Significant influence of the +93 C/T polymorphism in the apolipoprotein(a) gene on Lp(a) concentrations in the Asian Indian neonates from Singapore. *P.S Low[1], C.K Heng[1], S.C. Quek[1], N. Saha[2], M.I. Kamboh[2]*. 1) Dept Pediatrics, National Univ Singapore, Singapore, Singapore; 2) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburg, PA.

We studied the influence of the +93 *C/T* polymorphism in the apo(a) gene on plasma level of lipoprotein(a) [Lp(a)] in 282 South Asian Indian neonates using cord blood of both genders. Genotypes of kringle-4 (K4) and pentanucleotide repeats (PNR) polymorphisms were also available for adjusting their confounding effect on Lp(a) concentrations. The frequencies of the rare *T* allele was 0.15. The genotype distribution did not deviate significantly from a population at Hardy-Weinberg equilibirum. Significantly different mean Lp(a) levels were observed in descending order for the following genotypes *CC* > *CT* > *TT* ($F = 5.00$, $P = 0.007$) using 3-way ANOVA with K4 and PNR genotypes as the other 2 grouping factors. Our observation is in accordance to findings that the *T* allele was responsible for a reduction of apo(a) translation rate by 60% in vitro. The +93 polymorphism was able to explain 4.8% of the total variation in cord plasma Lp(a) levels. Based on a subset of 120 neonates with grandparental history of coronary artery disease, we found no significant difference in Lp(a) levels between neonates with family history and those without. Pearson's chi-square test was also not significant for cross tabulation of family history and +93 genotypes, indicating a similar genotype distribution between the two subgroups.

Genotype	Mean [lnLp(a)+1](SD)	Geometric Mean	*n*
CC	0.46 (0.52)	0.58	210
CT	0.23 (0.37)	0.26	63
TT	0.15 (0.14)	0.15	9

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Program Nr: 1158 from the 1999 ASHG Annual Meeting

Genetic polymorphisms in Transforming Growth Factor-b1 and the risk of Alzheimer's Disease. *E.K. Luedecking[1], S.T. DeKosky[1,2], H. Mehdi[1], M.I. Kamboh[1,2].* 1) Departments of Human Genetics; 2) Psychiatry, Univ Pittsburgh, Pittsburgh, PA.

Alzheimer's Disease (AD) is a multifactorial disease with the possible involvement of several genetic components. Genetic studies have yet to determine all genes involved in the pathogenesis of AD. Transforming growth factor-b1 (TGF-b1) is a candidate gene for AD. It is a multifunctional cytokine whose overexpression has been shown to promote the deposition of amyloid-b peptide in senile plaques. The goal of this study was to investigate the association of three polymorphisms in TGF-b1 with the risk of AD. Two of the polymorphisms in TGF-b1 are located at positions -800 (G®A) and at -509 (C®T), and the third site is in the coding region at codon 263 (Thr®Ile). We screened DNA samples of 428 sporadic, late-onset patients and 421 controls by PCR based assays. There was no statistically significant difference in genotype or allele frequency distributions between cases and controls for the -800 and codon 263 polymorphisms (p=0.38 and p=0.60). The overall genotype distribution at the -509 site was statistically significant (p=0.017). The frequency of the -509/TT genotype was significantly higher in AD patients than controls (p=0.015). This suggests that the C®T mutation may alter the regulation of the TGF-b1 gene and could possibly explain the overexpression of TGF-b1 in AD patients. The functional role of the -509 polymorphism was tested by Dual-Luciferase Reporter Assay. We sub-cloned the -509 C and -509 T alleles in front of the firefly luciferase reporter gene in pGL3-basic vector and cotransfected with pRL-CMV vector containing *Renilla* luciferase gene as a control for transfection efficiency in COS-1 cells. The activity of each promoter allele was directly measured by the ratio of firefly luciferase activity to *Renilla* luciferase activity. We observed an allele-specific effect on promoter activity of the TGF-b1 gene. The level of luciferase produced by the -509 T promoter allele was 2.8-fold greater than that produced by the -509 C allele (p=0.010). This data suggest that the -509 polymorphism has a functional effect on the promoter activity of TGF-b1.

Program Nr: 1159 from the 1999 ASHG Annual Meeting

Modeling Gene-Gene Interactions in Complex Traits. *F. Macciardi, V.S. Basile, J.L. Kennedy.* Centre for Addiction and Mental Health (CAMH), University of Toronto, 250 College St., Toronto ON M5T 1R8, Canada.

Complex issues such as gene-gene interaction require advanced statistical techniques. Consider each of two genes A and B, positioned on different chromosomes and hence not in linkage disequilibrium, that have been shown to have an effect on a given trait or disease, and we are interested in evaluating the combined effect of these two genes on the phenotype in question. The gene A/gene B interaction is based on the finding that either gene per se increases the risk for the trait, measured as a quantitative trait, i.e. a continuous variable. Continuous dependent variables are usually analyzed using the Analysis of Variance, where independent factors are represented by genotypes at loci A and B. Within the ANOVA model, we can test the effect of a possible genetic interaction contrasting the joint vector representing the double "pathological" variants for genes A and B against the other possible vectors combining the alternative genotypes of A and B: in this way we investigate whether the combined effect of possessing a pathogenic genotype for both loci shows a larger than expected outcome for the dependent variable (i.e., the phenotype) as compared to the effect of possessing only one pathogenic genotype, either A or B. Indeed, we know (Frankel and Schork 1996) that a genetic epistatic and/or additive interaction is not revealed by marginal effects, equivalent to a statistical interaction in the ANOVA model, and thus a non-significant value for the A x B effect is not an appropriate test to exclude a genetic interaction. In a "real data" example for the loci CYP1A2 and DRD3 with a movement disorder phenotype, we illustrate how the two genes interact with each other, using a contrast model for epistatic interaction.
Frankel and Schork: Who is afraid of epistasis ? Nature Genetics 14:371-373, 1996.

Program Nr: 1160 from the 1999 ASHG Annual Meeting

DNA based testing for the identification of progressive pseudorheumatoid dysplasia carriers. *A.M Alkhateeb[1], J. Al-Alami[1], S.M Leal[2], H.E El-Shanti[1]*. 1) Jordan University of Science & Technology, Irbid, Jordan; 2) The Rockefeller University New York, NY, USA.

Progressive pseudorheumatoid dysplasia is a skeletal dysplasia affecting the articular cartilage causing joint swelling and stiffness with additional changes in the spine similar to spondyloepiphyseal dysplasia tarda. It is a rare autosomal recessive disorder with high prevalence amongst Arabs specially the Jordanian population. The gene for this disorder has been mapped to the long arm of chromosome 6 and then fine mapped to a 2.1 cM interval. This area is covered by 3 highly polymorphic trinucleotide microsatellite markers (ATA11D10; ATA56D06; ATA16B01). Three families from Jordan proved to be linked to the same locus without a common hapolotype for the three markers. Linkage to the same locus supports the idea of a homogeneous disorder and the absence of a common haplotype indicates no common ancestor genes were inherited in our patients. Individuals at risk from the three families who agreed to participate and wanted to know their carrier status were included in the study. The study involved genotyping with the 3 microsatellite markers using PCR based non-isotopic polyacrylamide gel electrophoresis all available family members. The genotypes of the individuals together with haplotype analysis are capable of identifying heterozygous status with 98% certainty. This figure will rise to 100% once the gene is cloned and the actual mutation is identified. We here present DNA based testing for carrier identification for an autosomal recessive skeletal dysplasia. This testing is a preliminary tool for heterozygote and homozygote identification to be used in premarital counseling, prenatal diagnosis and presymptomatic diagnosis.

Program Nr: 1161 from the 1999 ASHG Annual Meeting

Genetic research in pharmaceutical clinical trials: investigator participation and ethics committee review and approval. *W. Anderson[1], J. Arbuckle[2], T. Arledge[1], C. Brazell[2], S. Concannon[1], L. Foster[2], A. Hughes[1], A. McCarthy[2], M. Mosteller[1], S. Ray[1]*. 1) Clinical Genetics Division, Glaxo Wellcome Research and Development, Research Triangle Park, NC; 2) Clinical Genetics Division, Glaxo Wellcome Research and Development, Greenford, Middlesex, UK.

Glaxo Wellcome (GW) is a research-based company whose people are committed to fighting disease by bringing innovative medicines and services to patients throughout the world and to the healthcare providers who serve them. As part of the strategy of developing innovative medicines, GW has incorporated genetic research into several ongoing development programs. Since the impact of genetic variation on response to medicines (pharmacogenetics) is a new science, GW established standardized procedures, study documents, and educational materials in conjunction with bioethics experts for investigators, study subjects, and ethics committees. Participation in the genetic research studies is voluntary. To ensure that information was presented consistently, GW developed templates for a genetic research protocol and an informed consent document. A video was produced to help investigators and study subjects understand the nature of the genetic research, use of the DNA sample, and use of genetic results. To supplement the consent and subject video, brochures with general background information were created. As of May 31, 1999, 1412 of 3817 investigator sites around the world (or 37%) were participating in genetic research; 6118 samples have been collected. For 28 sites participating in various studies, ethics committee approval was not granted. Reasons for non-participation by investigators and non-approval by ethics committees will be discussed in detail.

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Program Nr: 1162 from the 1999 ASHG Annual Meeting

Inherited Connexin 26 mutations associated with non-syndromic hearing loss in the Israeli population. *K.B. Avraham[1], T. Sobe[1], S. Vreugde[1], H. Shahin[1,2], M. Kanaan[2], Y. Yaron[3], A. Orr-Urtreger[3], M. Frydman[4], M. Shohat[5]*. 1) Department of Human Genetics and Molecular Medicine, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel; 2) Life Sciences Department, Bethlehem University, Bethlehem, Palestinian Authority; 3) Genetics Institute, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel; 4) Genetics Institute, Haim Sheba Medical Center, Tel Hashomer, Israel; 5) Department of Medical Genetics, Rabin Medical Center, Petah Tikva, Israel.

Connexin 26 (GJB2) mutations lead to hearing loss in a significant proportion of all populations studied so far, despite the fact that at least fifty other genes are also associated with deafness. The entire coding region of connexin 26 was sequenced in hearing impaired children and adults in Israel in order to determine the percentage of hearing loss attributed to connexin 26 and the types of mutations in this population. Thirty-one percent of all tested harbored GJB2 mutations, the majority of which were 35delG and 167delT mutations. Several parameters such as age of onset, severity of hearing loss and audiological characteristics, including pure-tone audiometry, tympanometry, auditory brainstem response (ABR), and otoacoustic emissions were examined to establish whether genotype-phenotype correlations exist. All GJB2 mutations were associated with prelingual, but not postlingual, hearing loss. Severity ranged from moderate to profound hearing loss, with variability even among hearing impaired siblings. The study here has direct clinical ramifications for genetic counseling in the hearing impaired population, both in Israel and the rest of the world.

Program Nr: 1163 from the 1999 ASHG Annual Meeting

Development of a Multiplex ARMS™Test for the Detection of Polythymidine Splice-Site Variants in Intron 8 of the CFTR gene. *A.I. Bayliffe, S.J. Kelly, N.H. Robertson, S. Little.* Product Development, AstraZeneca Diagnostics, Northwich, Cheshire, ENGLAND.

In cases of cystic fibrosis (CF) involving class I and II CFTR mutations chronic pulmonary disease is observed at variable levels with chronic pancreatitis. However, some CF patients present with milder symptoms ranging from sinopulmonary problems and pancreatic sufficiency (PS-CF), to congenital bilateral absence of the vas deferens (CBAVD).

Polythymidine (polyT) splice-site acceptor alleles 7T, 9T, and 5T occur at frequencies in the general population of 85%, 10%, and 5% respectively, and impact the splicing efficiency of exon 9. The most inefficient splice variant, the 5T allele, has been identified as contributing to expression of CF and CBAVD phenotypes in individuals with certain CF genotypes.

We have developed a single-tube multiplex ARMS™ allele specific amplification test, which genotypes for 5T, 7T, and 9T variants. Genomic DNA is added to a reaction tube and polyT alleles are detected by ARMS™, with subsequent analysis by standard agarose gel electrophoresis. The presence or absence of diagnostic product bands of defined sizes indicate polyT allele status. The test was validated using a large panel of DNA samples and was shown to be very reliable. The procedure is simple to perform, and provides rapid results (turn around in half a day), which are easy to interpret. The test has application to the detailed diagnosis of CF and infertility. Information on distribution of polyT variants with respect to CF genotype will be discussed in the context of previously published results.

Program Nr: 1164 from the 1999 ASHG Annual Meeting

Molecular analysis in Brazilian cystic fibrosis patients reveals five novel mutations. *A.L.F. Bernardino[1], A. Ferri[1], M.R. Passos-Bueno[1], C.A.E. Kim[2], C.M.A. Nakaie[2], C.E.T. Gomes[3], N. Damaceno[4], M. Zatz[1]*. 1) Centro de Estudos do Genoma Humano,Departamento de Biologia, Universidade de S.Paulo, SP, Brazil; 2) Instituto da Crianca, Universidade de S.Paulo, SP, Brazil; 3) Departamento de Pneumologia, Escola Paulista de Medicina, UNIFESP, SP, Brazil; 4) Departamento de Pediatria, Santa Casa de Misericordia, SP, Brazil.

Cystic Fibrosis (CF) is the commonest severe autosomal recessive disorder in the Caucasian population, with an estimated incidence of 1 in 2500 live births and a carrier frequency of 1 in 25. The CFTR gene at chromosome 7q31 has 27 exons spanning 230 Kb and encodes a protein of 1480 aminoacids. More than 800 different mutations in the CFTR gene have been already reported to the Cystic Fibrosis Genetic Analysis Consortium so far, the most common of all being the DF508 mutation. We have analyzed 160 CF Brazilian patients diagnosed with CF (73% Caucasians and 27% of mixed Negroid, Indian and Caucasian background). Screening of mutations in 320 CF chromosomes was performed through SSCP/Heteroduplex analyses assay followed by DNA sequencing of the 27 exons and exon/intron boundaries of the CFTR gene. The frequency of CFTR variants of T tract length of intron 8 (IVS8 Tn) was also investigated. This analysis enabled the detection of 232/320 CF mutations (72.2%) and complete genotyping of 61% of the patients. The DF508 mutation was found in 48.4% of the alleles. Another fifteen mutations (previously reported) were detected: G542X, R1162X, N1303K, R334W, W1282X, G58E, L206W, R553X, 621+1GT, V232D, 1717-1GA, 2347 delG, R851L, 2789+5GA and W1089. Five novel mutation were identified, V201M (exon 6a), Y275X (exon 6b), 2686 insT (exon 14a), 3171 delG (exon 17a), 3617 delGA (exon 19). These results contributed to the molecular characterization of CF in the Brazilian population and to enhance our knowledge on genotype/phenotype correlation. In addition, the identification of the novel Y275X mutation allowed pre natal diagnosis in a high risk fetus. Supported by FAPESP, CNPq, PRONEX.

Program Nr: 1165 from the 1999 ASHG Annual Meeting

Challenges in Molecular Testing for connexin-26 Mutations in Children Referred for Non-Syndromic Deafness.
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Mutations in connexin-26 (Cx26) account for a majority of AR non-syndromic deafness. Testing for two common mutations (30delG and 167delT) is predicted to identify over 90% of hearing loss due to Cx26 in European and Ashkenazi Jewish populations, and is a cost effective approach to the diagnosis of the etiology of deafness in children. We have analysed Cx-26 mutations in eight patients referred for AR or sporadic sensorineural deafness from diverse ethnic backgrounds. As the frequency of 30delG and 167delT in some ethnic populations is unknown and since the Cx-26 gene is relatively small, we have taken the approach of direct gene sequencing to identify mutations. Two patients were homozygous for 30delG, one patient was heterozygous for 30delG/unknown and four patients were heterozygous for mutations other than 30delG and 167delT: 176-191del(16), V27I and V37I. Deletion 176-191del(16) was found in one patient and generates a frameshift, altering the amino acid sequence starting at codon 59 and introducing a premature stop codon. V27I was found in one patient, a child with two deaf siblings; and V37I was found in two patients, one of who was also heterozygous for 30delG. Mutations at V27 and V37 may have pathological significance since both are conserved residues. However, V27I and V37I have each previously been reported in one out of 192 alleles in random control samples, leading the authors to speculate that they represent polymorphisms (Kelley et al, 1998, AJHG 62:792). We have subsequently screened 120 alleles from random samples for both V27I and V37I: one was positive for V37I; none were positive for V27I. Thus, the significance of these mutations in our patients remains unclear, but suggests a pathogenic role in AR deafness. Gene sequencing has identified three out of eight patients whose deafness may be based on mutations in Cx-26 that would have been missed using mutation analysis for 30delG and 167delT alone. Although sequencing results must be interpreted with caution, direct DNA sequencing has the potential to identify a larger proportion of patients with Cx-26 related hearing loss, and provide families with a defined recurrence risk.

Program Nr: 1166 from the 1999 ASHG Annual Meeting

ATM mutation scanning from genomic DNA of ataxia-telangiectasia patients by [Detection of Virtually All Mutations]-SSCP (DOVAM-S). *C.H. Buzin[1], V.Q. Nguyen[1], C.Y. Wen[1], G. Nozari[1], A. Mengos[1], X. Li[1], J.S. Chen[1], F.K. Fujimura[1], R.A. Gatti[2], S.S. Sommer[1]*. 1) City of Hope National Medical Center, Duarte, CA; 2) UCLA School of Medicine, Los Angeles, CA.

The ATM gene, which is mutated in the autosomal recessive disease ataxia-telangiectasia (A-T), contains 66 exons spanning ~146 kb of genomic DNA and produces one of the largest known proteins. The size of the ATM gene and the lack of mutational hotspots has hampered the detection of all mutations. DOVAM-S is a modification of SSCP in which up to 20 segments of DNA are analyzed in a single lane under five different electrophoresis conditions, including various matrices, buffers, temperature, and additives. In previous blinded or prospective studies of hemophilia B and A patients, DOVAM-S detected 100% of 87 and 84 mutations in the Factor IX and Factor VIII genes, respectively. In this study, 17.5 kb of the ATM gene were screened in 70 segments (166-367 bp each) that included exons 4-65 (coding regions) and the adjacent intronic splice junctions. Segments were grouped into four lanes of 17 or 18 amplicons and electrophoresed under five conditions. Segments showing abnormal mobilities were sequenced; mutations were confirmed by reamplification and sequencing from genomic DNA. In a blinded analysis of DNA from 19 A-T patients, a total of 80 sequence changes (39 unique mutations and/or polymorphisms) were detected. All known sequence changes within the scanning region were found. In addition to the known changes, 2 new common polymorphisms (in multiple samples) and 5 rare DNA variants were detected. An additional 12 putative causative mutations were also detected: 4 nonsense mutations, 2 deletions resulting in frameshifts, 2 mutations at the initiation codon, 1 exonic splice junction mutation and 3 missense mutations. Most of the new mutations are predicted to truncate the protein; thus, few missense mutations produce the A-T phenotype. Studies are in progress to screen genomic DNA from 41 additional A-T patients.

Program Nr: 1167 from the 1999 ASHG Annual Meeting

Referrer and patient expectations and realisation of genetic consultation in 7 countries. *K. Challen, V. Baranov, C. Bartsocas, J. Burn, J-P. Fryns, E. Ginter, H. Harris, R. Harris, I. Nippert, R. Salonen, J. Schmidtke, LP. Ten Kate.*
Genetic Enquiry Centre, St Mary's Hospital, Manchester.

Under 25% of patients (425) could expect ongoing care from a clinical geneticist in our study of 1794 referrals to 9 genetic centres in 7 countries. It is therefore important to consider referrer and patient expectations of their "snapshot" encounter with the genetic centre. Most referrers (66%) specifically requested specialist counselling; significantly more GPs and significantly fewer self-referrals ($p<0.001$). 733 (41%) referrers requested a clinical diagnosis, although this was lower in self-referred patients and those from GPs and obstetricians ($p<0.001$), perhaps reflecting increased rates of self and GP referral because of family history. For example, there was a significantly lower expectation of clinical diagnosis in patients whose eventual diagnosis was hereditary cancer ($p<0.001$). In only 74 (4%) cases did the geneticist consider that the patient's reasons for attending differed from the referrer's, despite only 24 (1%) having received a home visit before the genetic consultation. 520 (29%) patients were assessed purely clinically; significantly fewer amongst GP-referred patients ($p<0.001$) and, interestingly, significantly more amongst those with a final diagnosis of hereditary cancer ($p<0.001$). 721 (40%) patients had cytogenetic tests; significantly more amongst obstetric referrals, presumably reflecting high rates of referral for prenatal diagnosis. Of the 1186 (66%) cases in which a provisional diagnosis was given by the referrer, 693 (58%) were modified at the genetic centre. The likelihood of diagnostic modification varied widely between referrers, being significantly lower in GP and obstetric referrals. The final status of the diagnosis (certain, likely or uncertain) also varied widely between referrers, with significantly higher rates of certainty for obstetric referrals and significantly lower rates for GPs ($p<0.001$). It would seem that the service required from a genetic centre is affected greatly by its referrer customer base, and therefore that due account must be taken of health service structure in the planning and development of genetic services.

Program Nr: 1168 from the 1999 ASHG Annual Meeting

Typical Hispanic Population with Nontraditional Barriers. *S.M. Chenevert, H.A. Northrup, A.J. Tucker.* The University of Texas, Medical School Houston, TX.

Charts of 600 prenatal genetic counseling patients from a primarily Hispanic population (86%) receiving care in 6 outreach clinics in Houston, Texas were analyzed. Our goal was to identify barriers for acceptance of prenatal testing and diagnosis and thereby improve counseling through a better understanding of the population. Typical barriers already identified in the literature include: low socio-economic status, language, Catholic religion, and foreign born vs US born. Analysis of our population reveals low socioeconmic status (~60% qualify for public assistanc with the majority of remaining qualifying for Medicaid), Spanish speaking (seen by bilingual counselor), 34% Catholic (49% unspecified), and 83% foreign born. On average, 1/3 of our patients accepted prenatal testing of some form (high resolution U/S, amniocentesis, etc) but only 16.6% accepted an amniocentesis specifically. Additional data gathered includes: average age of mother, gestational age (GA) at time of GC visit, reason for referral, citizenship status, religion, ethnic background, acceptance of testing vs. follow through and specific characteristics of those who chose amniocentesis. From our analysis we can eliminate the most commonly described barriers of language, religion and being foreign born as the major barriers to our population. A lack of follow through with testing decisions was observed. Potential reasons include the amount of paperwork required to receive public assistance and the late gestational age (18.5 weeks) at the time of their first appointment with the genetic counselor (GC). To determine a causative factor for later referral, we analyzed time between referral and GC appointment (~2 weeks) giving an average GA of ~17 at time of referral. This is a significant barrier in light of 1) spacing of clinics (7-31 days depending on the clinic) and 2)the wait for prenatal diagnosis appointments. Our findings reinforce the need to educate both the population and physicians regarding the importance and availability of GC services. It is important to respect the heterogenity among Hispanic populations. Our data demonstrate that cultural stereotyping should be avoided.

Program Nr: 1169 from the 1999 ASHG Annual Meeting

Factor IX gene mutations in Korean hemophilia B patients. *Y.H. Cho, C.H. Lee, S.H. Shim, H.K. Seo.* Dept Medical Genetics, Hanyang Univ Col Medicine, Seoul 133-791, Korea.

Hemophilia B is an X-linked recessive bleeding disorder, resulting from mutations of the coagulation factor IX gene. Hemophilia B is known as to be caused by a variety of mutations, which can be found in the whole coding regions. The purposes of this study were to find out molecular defects of factor IX gene in Korean hemophilia B patients, and to establish a strategy for prenatal and carrier diagnosis. The promoter region, 8 exons and their intron boundaries of factor IX gene in genomic DNAs of 15 Korean hemophilia B patients were amplified by PCR, and this PCR products were screened for the mutations with the methods of SSCP(single strand conformation polymorphism) and BESS T-scan. And DNA sequence analysis was performed by subcloning of PCR product and dideoxy reactions. With SSCP analysis, 9 cases showed mobility shifts. Three cases out of the remaining 6 cases showed abnormal band patterns in BESS T-scan. Eighty percents (12/15) of mutation screening rates were achieved. One point mutation was detected in one cases of each at exon B, C, E and F, respectively, and in 2 cases at exon G. And 6 cases at exon H. In DNA sequence analysis, all of 12 detected mutations showed sequence alterations. Five cases were transversion, and 8 cases were transition, and 4 cases of transitions were on CpG dinucleotide sites. Nine cases were missense mutations and 3 cases were nonsense mutations. Five mutations were known by previous studies, and 7 cases were novel mutations found in this study. This study showed that the mutations in factor IX gene of Korean hemophilia B patients are extremely heterogeneous and wide spread over entire coding regions, although majorities of mutations showed a tendency to aggregate on exon H. In this study, a strategy for direct method of prenatal and carrier diagnosis for hemophilia B was established, and it will be useful to use this direct method as complementary to indirect linkage analysis.

Program Nr: 1170 from the 1999 ASHG Annual Meeting

The analysis of newborn dried-blood-spot specimens for cystic fibrosis mutations using PCR/OLA and capillary electrophoresis. *H. Chow, J.W. Eastman, R. Wong, J.E. Sherwin.* Gen Disease Lab, California Dept Hlth Services, Berkeley, CA.

Cystic Fibrosis (CF) is a common autosomal recessive disorder that can be lethal in childhood unless good continuous medical care and nutritional enhancement are provided early in life. To evaluate newborn screening in California for CF, the Genetic Disease Laboratory examined an in vitro assay (PE Applied Biosystems) that screened samples for the presence of 31 prevalent mutations by oligonucleotide ligation assay (OLA) and capillary electrophoresis. Two hundred and ninety-six newborn heelstick dried-blood-spot (DBS) specimens collected in 1987, 1995 and 1997 from all areas throughout the State were analyzed. A 3mm-diameter disk (3 µL of blood) was used for genomic DNA extraction. Multiplex PCR with 15 pairs of primers was used for amplification. The primers flanked regions (exons 4, 7, 9, 10, 11, 20 and 21, intron 17) of the gene where the mutations may be detected. The OLA that followed PCR provided sequence-coded electrophoretic mobility and fluorescent labeling for identification of the 31 allelic CF genotypes. An internal size standard was added to each sample for size measurement. The PE ABI Prism 310 genetic analyzer was used for the automated capillary electrophoresis. The capillary was fused silica cut to 30cm long, with inner diameter 75m. Linear polyacrylamide gel was used so that it would rinse out after each samples electrophoresis. Of the 296 newborn samples, none was homozygous. There were five heterozygous mutations, one I507, three F508, and one R117H. This gives a carrier frequency of 1 in 59, an expected result for the heterogeneous California population. The assay is an efficient method for identifying the 31 CF mutations. Pipetting for PCR and OLA are manual, the electrophoresis is automatic. For a 48-sample tray, twenty-four hours is the approximate analysis time. For high-throughput newborn screening, we are exploring a two-tiered approach in which immunoreactive trysinogen (IRT) is used to identify specimens for DNA analysis. A typical screening laboratory can test 250 DBS specimens for IRT, and analyze five specimens with the highest IRT results by the PCR/OLA capillary electrophoresis method, with one-day turnaround time.

Program Nr: 1171 from the 1999 ASHG Annual Meeting

Data collection in the Great Plains Genetics Service Network. *C.A. Christianson[1], P.S. Ing[2], J.M. Smith[3].* 1) University of South Dakota, Rapid City, SD; 2) Boys Town National Research Hospital, Omaha, NE; 3) University of Iowa College of Medicine, Iowa City, IA.

The goals of the GPGSN Database Committee were to: 1) describe demographically the patients seen for counseling & evaluation (C&E) & prenatal (PND) services in the region, and 2) provide a list of patients by diagnosis. In 1984, a system was designed to collect pre-patient-per-visit data on the delivery of genetic services (not diagnoses, not prevalence or incidence of genetic diseases). The C&E minimum data set (MDS) included 26 items. There were 21 items in the PND MDS.

A review of the data collected since 1987 suggests we were unsuccessful in completely accomplishing either goal. Although the most consistent data were collected between 1994 & 1997, inclusively, only 7 items in the C&E MDS and 10 items in the PND MDS had known valid data of >90%.

For the C&E patients: we were consistently unable to collect data related to socioeconomic status & diagnosis; 70% were under the age of 10; there were more males (56%) age 10 or younger and more females (67%) ages 20 to 40; 57% were new referrals & the most common reasons for referral were an abnormal phenotype & follow up.

For the PND patients: we were consistently unable to collect data related to pregnancy outcomes; most were referred for AMA or an abnormal maternal serum screen; 90% were referred by private physicians; diagnostic ultrasounds, amniocentesis and AFAFP were the most common tests performed; the racial distribution of PND patients reflected the regional birth statistics, and 54% were under the age of 35.

We conclude that a successful system should include: (1) adequate financial resources (personnel, hardware, software) at the center level to provide consistent data & at the regional level to analyze the data and provide timely technical assistance to the centers; (2) clear goals; (3) dedicated, focused, & expert leadership. Supported in part by grant MCJ-191002, Great Plains Genetics Service Network.

Program Nr: 1172 from the 1999 ASHG Annual Meeting

Palm-print on a sticker as a replacement of the blood for the DNA tests. *Y. Chung, C. Hwang, E. Kim.* I. D. Gene, Inc, Seoul, Korea.

DNA is omnipresent as far as cells could be traced. However, most of DNA samples are obtained from the blood for routine analysis. Blood-drawing is painful, potentially prone to infection, and requires special legal obligations for handling. We developed the skin as an alternative source of DNA for routine DNA tests. Examinees leave palm-prints on a sticker. From only a tenth of a square inch of the palm-print, we were able to obtain enough DNA for the DNA profiling using multiplex PCR systems. DNA extracted from the sticker showed exactly the same sequence as that obtained from the blood for the examined genetic regions including D8S306, TH01 and ACE gene. The DNA profiles for 13 STR loci matched exactly those of the blood DNA in 25 examined human samples. The palm-print DNA was successfully applied for paternity determination for over 30 cases during the past several months in our company. The examinees just made palm-prints on the delivered sticker and returned them to the laboratory like any ordinary letter. The DNA on the sticker was very stable that we could not detect any differences at least from a 9-month-old DNA. Furthermore, the fingerprint left on the sticker could be visualized by staining with crystal violet and identified the source of the DNA eliminating the need for the witness for the sample collection. The palm-print DNA on a sticker could be a convenient medium for most kinds of routine DNA tests and for academic research as well as for the DNA banks.

Program Nr: 1173 from the 1999 ASHG Annual Meeting

Identification of a new CFTR gene deletion by a real-time quantitative PCR method. *B. Costes[1], E. Girodon-Boulandet[1], D. Vidaud[2], E. Flori[3], P. Conteville[1], P. Fanen[1], F. Niel[1], M. Vidaud[2], M. Goossens[1]*. 1) INSERM U468, Hopital Henri-Mondor, Creteil, France; 2) Laboratoire de Genetique Moleculaire, Faculte de Pharmacie, Paris, France; 3) Service de Cytogenetique, Hopital de Hautepierre, Strasbourg, France.

We describe a novel CFTR gene deletion identified by an original approach of quantitative PCR using real-time detection, in a fetus from a turkish consanguineous couple, who presented with an hyperechogenic bowel. The use of the CF-OLA kit (PE-Biosystems), detecting 31 mutations, led us to suspect a deletion at the homozygous state in the fetus. A deletion of exon 19 was confirmed by quantitative PCR making use of the fluorescent TaqMan methodology (PE-Biosystems). The parents decided to continue the pregnancy despite the probable diagnosis of CF, which was confirmed after birth on the basis of positive sweat tests. RT-PCR analysis of CFTR mRNA of placental cells exhibited a shorter transcript than the wild type, corresponding to an in frame deletion of exon 19. Long range PCR experiments and subsequent direct sequencing showed a 5.3kb deletion removing exon 19, termed 3600+15kb del5.3kb (del19), with a direct AACT motif at the breakpoints. Immunoprecipitation experiments performed with the mutant transiently expressed in HeLa cells revealed a non matured 140 kDa form, whereas the wild-type CFTR exhibited a fully processed 170 kDa form. The mutant protein is thus predicted to be not expressed at the apical membrane of epithelial cells, accounting for the CF phenotype. Prenatal diagnosis is now possible for future pregnancies of the couple, if requested, as well as carrier testing in the family. This case illustrates the power of the real-time quantitative PCR approach in the rapid diagnosis of CF. This methodology should be offered as a new molecular tool in diagnosis of CFTR deletions.

Program Nr: 1174 from the 1999 ASHG Annual Meeting

Preliminary studies before systematic screening of hemochromatosis : prevalence and penetrance of C282Y homozygosity. *V. David[1], R. Moirand[2], CNAM St Brieuc[3], CNAM Rennes[4], CNAM St Nazaire[5], J.Y. Le Gall[1], Y. Deugnier[2], A.M. Jouanolle[1]*. 1) Fac de Medicine, UPR 41 CNRS, Rennes, Cedex, France; 2) Clinique des maladies du foie, CHU Pontchaillou, Rennes, France; 3) CNAM St Brieuc, France; 4) CNAM Rennes, France; 5) CNAM St Nazaire, France.

Hemochromatosis is considered as one of the most frequent inherited disorders in Caucasians and its systematic screening may be advocated. In Northern Europe, because most cases of hemochromatosis are related to homozygosity for the C282Y mutation on the HFE gene, genetic testing might be proposed for this general screening. However, before launching such a program in general population, it is mandatory to assess the prevalence and the penetrance of C282Y homozygosity. We tested the C282Y mutation frequency in a cohort of 1000 newborns from maternity hospitals in 4 different parts of Brittany. The homozygote frequency was 5/1000 and heterozygote frequency was 12%, which corresponds to higher frequencies than that expected from previous phenotypic studies. Moreover we are achieving a large survey of 10.000 subjects (men aged from 25 to 40 years and women aged from 35 to 50 years) from 3 different Breton health care centers. C282Y testing is performed in all subjects, and serum iron indexes (serum iron, transferrin saturation TS and ferritin) are measured in the first thousand people tested and in all subjects detected as C282Y homozygotes. Based upon wild C282Y homozygotes, upper normal of values have been calculated for serum iron indexes. Among the first 3017 subjects tested, 443 were found heterozygotes (14.7%), and 18 homozygotes for C282Y. All men had increased iron indexes (TS 50-92%) consistent with hemochromatosis. 6 out of the 12 women presented with elevated TS (49-98%) and the others had no phenotypic expression of the disease in the absence of pathological blood loss. Phenotypic expression was not relevant to age or genital status. These preliminary results (i) confirm the high frequency of genetically defined hemochromatosis (C282Y homozygosity), and (ii) suggest that the penetrance of the C282Y mutation is incomplete and modulated by either environmental or additional genetic factors.

Program Nr: 1175 from the 1999 ASHG Annual Meeting

Follow-up of mexican patients with genetic diseases at the National Institute of Pediatrics. *V. del Castillo, N. Urraca, C. Esmer.* Resarch Department of Human Genetics, Instituto Nacional Pediatria, SSA, DF, Mexico.

Children with genetic diseases must be followed-up by long periods of time in order to watch for new findings or just waiting to be diagnosed. Some of them do not return to receive medical attention which may have serious consequences. We analyze the patients' attendance to the genetic consultation, investigate some of the causes of stop seeking advice and determine the differences between those seeing for the first time at the office and those seeing while being hospitalized. Four hundred records were evaluated from 1992 and 1996. Our results show a mean follow-up period of 8.3 months (range 0-79) and the average of times on consultation was 2.8 times (range 1-16). Forty-eight percent of hospitalized patients were evaluated only once and 14%, twice. Of the 300 children analyzed from the outpatient consultation, 22% and 21% attended once and twice respectively, showing significant differences between hospitalized versus office patients (p=0.00). The patient's diagnosed status didn't affect the follow-up. 97 patients were discharged, 7 died, 55 continued being followed by us, 62 attended to other services at the hospital but not ours and 179 didn't assist at all. Diagnosed patients received genetic counseling more frequently than undiagnosed patients did (62% vs 5%). On the diagnosed group genetic advice was given to 65% and 78% of the office patients in 1992 and 1996 respectively (Not significant); 23% and 50% of the hospitalized patients on the same years (p=0.04), and the differences between this two groups were significant (p=0.005). We conclude that keeping the genetic patient on follow-up is a difficult task, sometimes before receiving genetic advice. New educational strategies must be planned to avoid this worrisome situation.

Program Nr: 1176 from the 1999 ASHG Annual Meeting

Microsatellite DNA Analysis Using High Performance Liquid Chromatography. *J.M. Devaney[1], J.E. Girard[2], M.A. Marino[1]*. 1) Transgenomic, Inc., Gaithersburg, MD 20878; 2) American University, NW Washington, DC 20016.

The genetic uniqueness of individuals is a central tenet of human biology. One method to examine the uniqueness of an individual is the use of microsatellite or short tandem repeat (STR) length polymorphisms. STRs are tandemly repeated units of sequence ranging between 2 and 7 basepairs in length that have high levels of heterozygosity, distinguishable alleles that are length polymorphic as a result of the number of repeating units, and are amplified using the PCR reaction. STR systems are useful for disease diagnostics (Fragile X, Huntingtons disease), paternity testing, physical and genetic mapping, cancer detection, and forensics. The human genome has an abundance of these sequence occurrences averaging one tri- or tetra- nucleotide repeat unit every 15 kb. Analysis of STRs using slab gel electrophoresis requires lengthy and labor-intensive procedures. An alternative to electrophoresis is ion-pair reverse-phase high performance liquid chromatography (IP-RP HPLC). The WAVE Nucleic Acid Fragment Platform (Transgenomic, Inc.) employs IP-RP HPLC, which has been shown to give strict and reproducible size-based separation of DNA fragments up to 2,000 bp when run in a non-denaturing mode. The system is also automated, which allows for unattended analysis of 96 samples directly from the PCR plate. Another distinct advantage of IP-RP HPLC is that resolved products can be collected for subsequent analyses such as sequencing. The following discussion describes the use of WAVE technology in the separation, sizing, and typing of alleles from the locus HUMTHO1 located in intron 1 of the human tyrosine gene with a chromosomal position of 11p15-15.5. The alleles were sized in their native state (double stranded). The repeating unit of HUMTHO1 is (AATG) and the alleles are numbered according to the number of tandem repeats present in the PCR-amplified products. This non-electrophoresis platform discriminated the six alleles of THO1 with a resolution greater than 3. This locus is widely used in forensic and parentage assessment cases, therefore it serves as a great model for microsatellite sizing and typing.

Program Nr: 1177 from the 1999 ASHG Annual Meeting

Development & validation of ELUCIGENE™ reagents for CF screening. *E. Docter, A.J. Gladwin, S.L. Weston, N.H. Robertson, S. Little.* AstraZeneca Diagnostics, Northwich, Cheshire, UK.

Screening for cystic fibrosis is becoming more widespread and there is a need for a cost-effective method which can reliably detect many mutations. Here we report on the development and validation of multiplex ARMS™ allele specific amplification reagents which combined detect 20 CFTR mutations selected on the basis of their reported prevalence in the Caucasian European population. The primary application of these reagents is screening for individuals who may be carriers of one of the following mutations: 1717-1G>A, G542X, W1282X, N1303K, DF508, 3849+10kb C>T, 621+1G>T, R553X, G551D, R117H, R1162X, R334W, A455E, 2183AA>G, 3659delC, 1078delT, DI507, R347P, S1251N and E60X. In addition these reagents generate genotype information for the most common mutation, DF508, thus making the test highly valuable in both disease diagnosis and screening applications.

Genomic DNA is added to 3 tube multiplex reactions, amplified by PCR and resultant reaction products separated by agarose gel electrophoresis. Detection is based on the presence or absence of a diagnostic band of a defined size indicating whether or not a particular mutation is present in the sample. This rapid and simple procedure, which requires minimal equipment, can be carried out in less than one day and in contrast to alternative methods requires no subsequent manipulation after amplification other than gel loading. The reagents were validated on a panel of over 250 previously typed samples, including clinical samples collected and extracted by a variety of different methods and was shown to be completely reliable.

Program Nr: 1178 from the 1999 ASHG Annual Meeting

BRCA testing among non-geneticist physicians: Level of knowledge and the extent to which they are discussing and ordering testing. *T. Doksum[1], N.A. Holtzman[2], B.A. Bernhardt[2]*. 1) Abt Associates Inc., Cambridge, MA; 2) Johns Hopkins Medical Institutions, Baltimore, MD.

As part of a 1998 study to assess the diffusion of genetic testing among non-geneticist physicians, we surveyed 2,250 oncologists, Ob-Gyns and internists about 1) their knowledge about BRCA testing and 2) whether or not they had ever discussed and/or ordered BRCA testing. We hypothesized that physicians who had discussed or ordered the test would be more knowledgeable compared to those who had not. The overall response rate to the mail questionnaire was 40%. Only 13% of internists, 21% of Ob-Gyns, and 40% of oncologists were able to correctly answer all four basic knowledge questions. The majority of oncologists (84%) and Ob-Gyns (72%) but only one-fourth of internists (28%) reported that they had ever discussed BRCA testing with any patient. Few respondents had ever ordered a BRCA test, and usually did so through a genetics professional (29% of oncologists, 15% of Ob-Gyns, 10% of internists) rather than directly from a laboratory (15% of oncologists, 6% of Ob-Gyns, 1% of internists). Among oncologists, knowledge was significantly higher among those who had ever discussed the test ($p<.01$) or ordered the test either directly or indirectly ($p<.01$) compared to those who had not. Among Ob-Gyns and internists, knowledge was not significantly associated with having discussed or ordered the test. These findings suggest that, despite low levels of knowledge about BRCA testing, many non-geneticist physicians have discussed testing for breast cancer susceptibility and some have directly ordered testing.

Program Nr: 1179 from the 1999 ASHG Annual Meeting

Genetic Evaluation of Male Factor Infertility. *T.M. Dunn[1], M.M. McGovern[1,2], N. Bar-Chama[3], N.B. Kardon[1,2].* 1) Dept Human Genetics, Mount Sinai Medical Ctr, New York, NY; 2) Dept Pediatrics, Mount Sinai Medical Center, New York, NY; 3) Dept Urology, Mount Sinai Medical Center, New York, NY.

The evaluation of couples with primary infertility has revealed that male factors are present in 50% of cases. With the widespread availability of Assisted Reproductive Techniques, it has become extremely important to evaluate males for genetic etiologies of infertility including chromosome abnormalities, Y chromosome microdeletions, and single gene disorders. Our center began a formal comprehensive male factor infertility genetic evaluation program in July 1997 which includes genetic consultation and pedigree analysis, routine chromosome analysis, PCR screening for Y chromosome microdeletions, and cystic fibrosis (CFTR) mutation analysis, including the 5T variant. To date we have evaluated 100 patients who presented with either azospermia or oligospermia. The results of the genetic laboratory tests identified 4 patients with chromosome abnormalities, three patients with Y-chromosome deletions, two patients homozygous for 2 different cystic fibrosis mutations, and five patients heterozygous for one cystic fibrosis mutation. Pedigree analysis identified one patient with Kallmann syndrome and another patient heterozygous for Spinal Muscular Atrophy, Type 1. Overall, a genetic etiology for male infertility was identified in 16% of men studied. Since the most common abnormality detected in our population was a cystic fibrosis mutation, it's extremely important to screen partners to assess reproduction risk before considering newer reproduction options, ie. ICSI, in order to provide prenatal diagnosis options. The risk of transmitting the Y chromosome microdeletions to male offspring is also an issue that should be considered by couples considering ICSI. Based on the high rate of genetic etiologies detected in our series we recommend that male factor infertility patients have a complete genetic evaluation including pedigree analysis, a specific CFTR panel, chromosome analysis and PCR screening for Y chromosome microdeletions. Follow-up genetic counseling of all patients found to have a genetic etiology is also necessary to ensure that appropriate screening of partners is achieved.

Program Nr: 1180 from the 1999 ASHG Annual Meeting

Clinical diagnostic testing for Presenilin-1 (PS-1) gene mutations and diagnosis of early-onset familial Alzheimer's disease (EOFAD). *K.C. Fafel[1], U.A. Ananth[1], J.G. Jones[1], J. Elles[1], S.K. Allen[1], Y. Liang[2], Y-Q. Song[2], P. St. George-Hyslop[2], W.K. Seltzer[1], M.A. Boss[1]*. 1) Athena Diagnostics, Inc., Worcester, MA; 2) Centre for Research in Neurodegenerative Diseases, Toronto, Ontario.

The PS-1 gene on chromosome 14 is known to be the major gene for autosomal dominant early-onset familial Alzheimer's disease (EOFAD). Studies have shown that a variety of PS-1 mutations distributed throughout the gene are responsible for an estimated 30-50% of EOFAD. Clinical diagnostic testing for PS-1 gene mutations at Athena employs a sequencing strategy utilizing genomic DNA as template for exon 4 and cDNA for exons 5 through 13. Mutation identification in the entire coding region of the PS-1 gene (1,404bp) is carried out by sequencing in both the forward and reverse directions. RNA and DNA specimens analyzed from 242 patients detected 20 different mutations in 34 individuals. The previously reported Glu318Gly polymorphism was found in eight additional patients. Mutations identified consisted of 18 missense (17 heterozygous and 1 homozygous), one 6bp insertion and one deletion, distributed in exons 5, 6, 7, 8, 9, 10 and 13. Neither the insertion nor the exon 10 deletion mutation disrupted the reading frame of the protein. The overall positive rate of the PS-1 test was 14% with previously unreported mutations accounting for 70% of total mutations detected. Three of the previously unreported missense mutations were found to reoccur in apparently unrelated families. Interestingly, we identified individuals with two missense mutations in siblings from a single family. No nonsense or synonymous codon mutations have been detected to date. The median age of PS-1 mutation positive patients at time of testing was 44 years (range 31 to 65 years) with the exception of one symptomatic individual who was 78. Thirty three out of the 34 positive patients referred for PS-1 mutation analysis were reported to be symptomatic at time of testing. Our experience with clinical diagnostic testing for PS-1 mutations indicates that a substantial proportion of individuals presenting with EOFAD possess identifiable mutations leading to confirmation of disease diagnosis.

Program Nr: 1181 from the 1999 ASHG Annual Meeting

Fragile X syndrome: Population carrier screening and implication for prenatal diagnosis. *T.C. Falik-Zaccai[1], E. Shachak[1], Z. Borochowitz[1], N. Magal[2], S. Zatz[2], M. Shochat[2], H. Ziv[3], R. Navon[3], C. Ligum[4], R. Shomrat[4].* 1) Simon Winter Inst Human Gen, Bnai-Zion Med Ctr Haifa, Israel; 2) Genet. Inst. Shnieder Med Cent Petach Tikva, Israel; 3) Meir Med Cent Kfar Sava, Israel; 4) Genet. Inst. Soraski Med Cent Tel Aviv, Israel.

Fragile X syndrome (Fra) is a common cause of learning disability and mental retardation (MR). Identification of women carriers of the common premutation or full mutation in the FMR1 gene is mandatory for accurate genetic counseling and prenatal diagnosis. We have conducted a screening program between 1992 and 1997 for the detection of women carriers of the Fra. Women were offered testing on a self pay basis during routine prenatal visits or were referred by their physician, nurses or friends. 9426 healthy women with no family history of MR or learning disability requested the testing as well as 404 women with a family history of undefined MR. Screening was performed by Southern blotting for the presence of premutation or full mutation alleles followed by confirmation of their size by PCR when possible. We have found 111 women carriers of alleles of more then 52 CGG repeats among the group of women with no family history of MR, a prevalence of 1/85. Three full mutation alleles were identified in this group, a prevalence of 1/3145. Among the group with positive family history of MR 7 premutation alleles and 1 full mutation allele were found, a prevalence of 1/58 and 1/404 respectively. The identified carriers were offered genetic counseling and prenatal diagnosis. All carriers elected to perform prenatal diagnosis. Expansion of the CGG repeat size in the fetuses was documented for alleles of 49 repeats and above. Expansion of CGG repeat size to a full mutation was documented for alleles greater then 70 repeats only. These results indicate that the prevalence of carriers of Fra is high in Israel and a comprehensive national screening program should be considered. The DNA testing for carrier status was accepted by most women approached and requests for such testing are more common todate. When accompanied by genetic counseling and prenatal diagnosis it has established an important addition to current medical practice.

Program Nr: 1182 from the 1999 ASHG Annual Meeting

Discrimination of Diagnostic Criteria for Testing for BRCA1/2. *M.H. Fries[1], C. Holt[2], I. Carpenter[1], L. Martin[2], R. Hume[2], J. Flanagan[1], J. Daniels[2], G. Hudson[1], M. Cadman[1], R. Weatherly[1], M.E. Nunes[1]*. 1) Air Force Medical Genetics Cen, Keesler Medical Center , Keesler AFB,MS; 2) Madigan Army Medical Center, Ft. Lewis, WA.

The DOD Familial Breast/Ovarian Cancer Research Project has offered genetic counseling and testing for BRCA1/2 mutations on a research basis to patients who meet specific diagnostic criteria: patients with breast/ovarian cancer with 1) a family history of two or more 1st or 2nd degree relatives with breast cancer only *or* breast/ovarian cancer; 2) a 1st or 2nd degree relative with diagnosis of breast/ovarian cancer <45; 3)breast/ovarian cancer diagnosed <45; 4)bilateral breast cancer or other primary cancers; as well as 5)male patients with breast cancer; *and* 6)unaffected patients with a family member with a known BRCA1/2 mutation. Risk for BRCA1 mutation was calculated using the Couch model. 101 patients met criteria, including 33 who met criteria in more than one category (personal age of onset of cancer <45 + strong family history; personal age of onset of cancer <45 + other primary cancers; personal history of cancer and family history of 2 or more 1st/2nd degree relatives with onset of disease <45, and history of bilateral disease and/or multiple primaries + strong family history). 90 patients chose to test. In this group, mutations were found in 14 patients(15.5%) and unclassified variants identified in 20 (22.2%). When criteria were compared, the most common indications for testing were onset of breast/ovarian cancer<45 (n=32) and onset of breast/ovarian cancer <45 + strong family history (n=21). However, when the number of mutations and unclassified variants were compared separately across all diagnostic criteria (including those with more than one category) using c^2test, no significant differences were seen among the categories to suggest that one criteria was more predictive of mutations or variants than another. Couch values for patients with mutations ranged from 3.2% to 43.5% (range for all patients:1.2% to 69.7%). These findings emphasize the importance of using multiple diagnostic criteria plus Couch risk values of >3% as possible managed care guidelines for patient testing.

Program Nr: 1183 from the 1999 ASHG Annual Meeting

Informative molecular analyses for over 90% of hemophilia A families by direct mutation detection. *F.K. Fujimura, C.Y. Wen, C. Buzin, V.Q. Nguyen, W. Li, S.S. Sommer.* City of Hope National Medical Center, Duarte, CA.

Identification of causative mutations within the factor VIII gene is critical for accurate evaluation of carrier status of at-risk females and for prenatal diagnosis in hemophilia A families. Our analysis of over 200 hemophilia A families resulted in detection of factor VIII gene sequence alterations in over 90% of these families. Initially, analysis is performed on a DNA sample from an affected individual or, if an affected individual is not available, from an obligate carrier. All samples from severe hemophilia A families are tested by a novel amplification method for the factor VIII gene inversion. These inversions are detected in about half of the cases of severe hemophilia A. Samples not having a factor VIII gene inversion are scanned for mutations, either by direct DNA sequence analysis or by rapid mutation scanning methods that permit cost-effective scanning of all 26 exons plus the associated splice junctions of the factor VIII gene. In a prospective analysis of 88 DNA samples we have shown that our mutation scanning method is as sensitive as DNA sequencing for detection of mutations in the factor VIII gene. Furthermore, mutation scanning has several advantages over full gene sequence analysis including higher throughput and lower costs. The samples not having detectable mutations may have factor VIII gene mutations located outside of the analyzed regions of the gene, may have mutations in the factor VIII binding site of the von Willebrand factor gene, or may be due to misdiagnosis. To date, we have not detected any von Willebrand Normandy mutations in samples from 18 hemophilia A families not having detectable mutations in the factor VIII gene. This tiered analysis starting with factor VIII gene inversion analysis, followed by mutation scanning of all exons and intron junctions of the factor VIII gene and, if necessary, analysis of von Willebrand factor Normandy mutations, permits detection of putatively causative mutations in at least 90% of these families. Appropriate individuals in these families with identifiable mutations, in turn, can be offered very accurate carrier analysis and prenatal diagnosis.

Program Nr: 1184 from the 1999 ASHG Annual Meeting

Autosomal recessive LGMDs in Spanish families: the mutational spectrum. *P. Gallano[1], A. Lasa[1], A. Saenz[2], C. de Diego[1], A. Cobo[2], E. del Rio[1], M. Urtasun[2], M.J. Rodriguez[1], E. Gallardo[1], J.J Poza[2], F. Garcia Bragado[3], I. Illa[1], A. Lopez de Munain[2], M. Baiget[2]*. 1) Hospital Sant Pau, Barcelona, Spain; 2) Hospital Ntra. Sra. de Aranzazu, San Sebastian, Spain; 3) Hospital Virgen del Camino, Pamplona, Spain.

Autosomal recessive limb girdle muscular dystrophy (LGMD2) is a clinically and genetically heterogeneous group of diseases characterised by progressive proximal limb muscle weakness. Seven different loci have been mapped and six genes have been identified: calpain 3 at LGMD2A (15q15), dysferlin at LGMD2B (2p12), four components of the sarcoglycan (SG) complex: -SG at LGMD2D (17q21), -SG at LGMD2E (4q12), -SG at LGMD2C (13q12) and -SG at LGMD2F (5q33). The gene product at LGMD2G (17q11) is still unknown. We have studied 182 families with a muscular dystrophy history from all over Spain in which a dystrophinopathy was excluded by genetic and immunochemical/Western blot tests. The immunochemical analysis with -, -, - and -SG antibodies allowed us to distinguish between sarcoglycanopathies and the other forms of LGMD2. The molecular studies were based on: 1) The identification of point mutations by SSCP analysis and sequencing, 2) Linkage analysis using different microsatellites markers. We have diagnosed 115 families: 79 LGMD2A, 2 LGMD2B, 23 LGMD2C, 8 LGMD2D and 3 LGMD2E. In the sarcoglycanopathies the observed phenotype varied from severe (DMD-like) to moderate with mutations spreading all over the SG genes. The C283Y mutation in the -SG gene was observed exclusivelly in the Gypsy population and was always associated with a severe phenotype. In the LGMD2A families a recurrent mutation in exon 22 (2362AG->TCATCT) of the CAPN3 gene was found in the Basque population associated with the classical phenotype described by Fardeau et al. The remaining mutations were distributed all over the gene and were associated to different degrees of phenotypic variability. The clinical and immunochemical studies do not allow to perform an accurate diagnosis of LGMDs. Molecular studies are the most reliable tool in order to classify each of the different entities.

Program Nr: 1185 from the 1999 ASHG Annual Meeting

Factors Influencing Decisions to Decline Cancer Genetic Counseling Services. *K.P. Geer[1], W.F. Cohn[2], M.E. Ropka[2], S.M. Jones[3], S. Miesfeldt[4].* 1) Virginia Kincaid Fellow, Division of Surgical Oncology, University of Virginia Health Sciences Center (UVA-HSC), Charlottesville, VA; 2) Department of Health Evaluation Sciences, UVA-HSC, Charlottesville, VA; 3) Cancer Center, UVA-HSC, Charlottesville, VA; 4) Division of Hematology/Oncology, UVA-HSC, Charlottesville, VA.

Introduction: Genetic counseling services continue to develop nationwide to provide risk assessment and management strategies to individuals at risk for inherited cancer predisposition. Despite a plethora of research on factors influencing choices related to genetic testing for cancer risk, limited data have been published on psychosocial and economic issues that lead individuals to decline cancer genetic counseling services. **Purpose:** To identify potential barriers to broad utilization of cancer genetic counseling services. **Methods:** Using a cross-sectional design, 77 subjects were randomly selected from 117 individuals who declined cancer genetics evaluation in our institution between May 1995-October 1998. Of the 54 individuals contacted, 37 (69%) participated in a semi-structured telephone interview. **Results:** Of the participants, 19 (53%) were college graduates; 34 (92%) were female; 36 (100%) were Caucasian; and 26 (72%) were between 36-55 years. Median income was $40,000-59,000. Twenty-four (65%) had only a family history of cancer; 13 (35%) had a combined family and personal history of cancer. Responses to the question, "Why did you decide not to have the cancer genetics evaluation?", were: *potential impact on insurability for self or family* (15); *cost* (12); *emotional impact on self or family* (11); *no perceived benefit* (11); *time involved* (9); *difficulty collecting family history* (4); *other* (17). **Conclusion:** To address barriers to utilization of cancer genetic counseling services, the following changes in policy and practice are recommended: legislation to protect individuals with genetic risk for cancer from insurance discrimination; coverage of cancer genetics evaluations by third party payors; individualized emotional support throughout the decision-making process; education on potential benefits for the individual and family members; and development of strategies to streamline the genetic evaluation process.

Program Nr: 1186 from the 1999 ASHG Annual Meeting

Single tube genotyping of the Apolipoprotein E gene by ARMS™ allele specific amplification. *A.J. Gladwin, S.L. Weston, N.H. Robertson, S. Little.* AstraZeneca Diagnostics, Northwich, Cheshire, UK.

Elevated levels of cholesterol, lipids and lipoproteins are known to increase the risk of atherosclerosis and coronary artery disease in humans. Apolipoprotein E (Apo E), an important component in lipoprotein metabolism, serves as a ligand for the uptake of lipoproteins from the circulation by the liver. It is also expressed in the nervous system in astrocytes. In the general population, Apo E can be present as three isoforms: Apo E2, E3 and E4. Homozygosity for the E2 genotype (E2/E2) is associated with type III hyperlipidemia.

We have developed a single-tube multiplex ARMS™ allele specific amplification test, which simultaneously genotypes for E2, E3 and E4 variants. Genomic DNA is added to a reaction tube and the Apo E genotypes are differentiated by *ARMS*, with subsequent analysis by standard agarose gel electrophoresis. The presence of diagnostic product bands of defined sizes indicate Apo E status. The test has been validated on a large panel of samples and has been shown to be very reliable. The procedure is more simple and rapid than conventional RFLP and PAGE analysis and results are typically available within 1/2 working day.

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Program Nr: 1187 from the 1999 ASHG Annual Meeting

Utility of ELUCIGENE™ MCAD reagent for detection of the 985A>G mutation of the medium chain acyl CoA dehydrogenase gene in neonatal bloodspot samples. *D. Halsall[1], J. Calvin[1], T. Elsey[1], A.F. Heeley[2], G. Ellison[3], N.H. Robertson[3].* 1) Department of Clinical Biochemistry, Addenbrooke's Hospital, Cambridge, UK; 2) Biochemical Genetic Diagnostic Unit, Peterborough District Hospital, Peterborough, UK; 3) AstraZeneca Diagnostics, Gadbrook Park, Northwich, UK.

Medium chain acyl CoA dehydrogenase deficiency (McKusick 201450) is the most common inherited defect of fatty acid metabolism. A point mutation (985A>G) accounts for approximately 90% of the disease-causing mutant genes in this condition (Yokota *et al.* Am J Hum Genet (1991) 49:1280-1290). This potentially fatal condition is amenable to treatment and thus is a candidate for neonatal screening. A test for use in these circumstances must be cost-effective, reliable, robust and suitable for the analysis of blood spot samples. We report on the utility of the ELUCIGENE™ MCAD reagent for detection of the 985A>G mutation. The test method is based on ARMS™ allele specific amplification. A cohort of 500 blood spots collected for routine biochemical screening in East Anglia was analysed. Genomic DNA prepared from the blood spot samples by ethanol extraction was added to reaction mix comprising ELUCIGENE™ reagent, followed by PCR and subsequent analysis of the amplification products by agarose gel electrophoresis. Interpretation of results was achieved by absence or presence of amplification products of a defined size. All steps were performed in a 96 well plate format to allow semi-automation of the process. The frequency of the 985A>G mutation was determined in this cohort to be 1:125 (95% confidence limit 1 in 49 to 1 in 458, Poisson distribution) and no homozygotes were detected. This frequency is similar to values contained in other UK regions (Seddon *et al.* Lancet (1995) 345:135-136).

Program Nr: 1188 from the 1999 ASHG Annual Meeting

Continuing care for genetic patients in 9 European centres. *H.J. Harris, V. Baranov, C. Bartsocas, J. Burn, K. Challen, J-P. Fryns, E. Ginter, R. Harris, I. Nippert, R. Salonen, J. Schmidtke, LP. Ten Kate.* Genetic Enquiry Centre, St Marys Hosp, Manchester.

Patients often need contining and coordinated care when they have genetic disorders affecting several systems and generations. Genetic service providers might be expected to recontact patients with news of advances in diagnosis and treatment and to ensure that relatives are aware of their inherited risk and screening and preventive options. However poor coordination of care is frequently cited by genetic patient organizations as a cause for concern. In our study the need for continuing care was reported in 857 (48%) of 1794 patients referred consecutively to 9 medical genetics centers in 7 European countries. One or more of the following were expected to provide continuing care: clinical geneticist 47.4%, obstetrician 31.9%, pediatrician 25.7%, GP 13.4%, surgeon 12.2% and "other" 30.5%. The clinical geneticist offered continuing care significantly more often when the diagnosis remained uncertain. Written reports were sent to patients (86.3%), referrer (77.4%) and GP (40.2%); the proportions did not differ significantly whether continuing care was needed or not. The need to contact the patient's family was reported in 312 (17%) overall but in 128 (66.6%) of 192 cases of hereditary cancer (breast, ovary, bowel, MEN). 84.4% of patients referred because of hereditary cancer agreed to have their families contacted (when relevant), 99% of patients made the first contact but only 17.7% had assistance from professionals. 42.4% hereditary cancers were detailed on genetic registers. In 97% of all cases collaborators reported that "all continuing care needs will be provided" but in the absence of a recall and recontact system it is difficult to assess the quality of continuing care after completion of the specialist geneticist episode. Resource limitations, ethical problems and wide variations in national health care systems have been shown to affect implementation (Concerted Action on Genetic Services in Europe, CAGSE).

Program Nr: 1189 from the 1999 ASHG Annual Meeting

Hereditary cancer referrals to genetic centres. *R. Harris, V. Baranov, C. Bartsocas, J. Burn, K. Challen, J-P. Fryns, E. Ginter, HJ. Harris, I. Nippert, R. Salonen, J. Schmidtke, LP. Ten Kate.* Genetic Enquiry Centre, St Marys Hosp, Manchester.

By late 1998 hereditary cancer accounted for 192 of 1794 (10.7%) consecutive referrals to 9 medical genetics centres in 7 European countries, making it the second commonest reason for referral after PND. Some centres had much higher frequencies (up to 35.4%), perhaps because of their special interests and the specialty of referrers. For example 12/13 (93.3%) referrals from oncologists concerned hereditary cancer and comparable figures from other specialists were surgeons 22/50 (44%) and gynaecologists 10/25 (40%), compared with obstetricians who showed the lowest frequency of such referrals, 12/516 (2%). But the greatest *absolute* number of referrals for hereditary cancer, 60 of 192 hereditary cancer referrals (31%) came from GP even though only 212/1794 (11.8%) of all referrals were from GP. The overall pattern of referral from GP differed very significantly from other referral sources ($P<0.001$). Genetic services need to adapt to molecular advances and to increasing public awareness in ways which are appropriate to the type of health care system of the country. Where GP acts as gatekeeper controlling access to specialists it appears that a higher proportion of high-profile genetic disorders will be referred directly to genetic clinics. In other countries primary care is provided by specialists who may feel more confident to deal themselves with hereditary variants of diseases in their specialty, although earlier studies by UK National Confidential Enquiry into Counselling by Non-geneticists were not completely reassuring on this point. The present study is continuing by assessing all 1794 referrals to the nine European centres including the quality of referrals and of continuing care in different specialties.

Program Nr: 1190 from the 1999 ASHG Annual Meeting

Sex determination of fetus by polymerase chain reaction using maternal plasma and serum. *H. Honda, N. Miharu, Y. Ohashi, N. Honda, K. Ohama.* Dept Obstetrics & Gynecology, Hiroshima Univ. School of Med., Hiroshima, Japan.

In order to assess the possibility of the fetal sex determination using maternal plasma and serum, detection of Y chromosome specific sequences was performed with polymerase chain reaction (PCR). Peripheral blood samples were obtained from 52 pregnant women at 10-17 gestationals' weeks prior to amniocentesis. For plasma separation, 4ml of each blood sample was collected into EDTA-containing tubes for plasma separation, and 6 ml of each blood sample was collected into EDTA-free tubes for serum separation. DNA was extracted from 0.8 ml of each plasma or serum with QIAamp blood kit. To detect Y chromosome specific sequences, DYS14 and DYZ3, 40 cycles of PCR was carried out with each DNA solution. The PCR products of DYS14 and DYZ3 are 198 bp and 175 bp, respectively, and they were analyzed by 2.5% agarose-gel electrophoresis and ethidium-bromide staining. The PCR results were compared to cytogenetic analyses of amniocentesis. Cytogenetic analysis of amniocentesis revealed that 27 pregnant women had a male fetus and the remaining 25 pregnant women had a female fetus. Both DYS14 and DYZ3 sequences were detected in plasma samples obtained from 23 of 27 pregnant women having a male fetus, while both DYS14 and DYZ3 sequences were detected in all serum samples obtained from 27 pregnant women having a male fetus. Neither DYS14 and DYZ3 sequences were detected from both plasma and serum samples obtained from all the 25 pregnant women having a female fetus.These data indicates that it is possible to determine fetal sex by PCR using maternal serum, and maternal serum and plasma would be useful and powerful materials for prenatal diagnosis in the future.

Program Nr: 1191 from the 1999 ASHG Annual Meeting

Does China needs its own Down's screening strategy? *X. Hou[1], F. Song[1], L. Zhang[1], G. Feng[1], B. Qi[1], J. Han[2]*. 1) Laboratory of Medical Genetics, ShenYang Maternal Child Care Hospital, Liaoning, P.R. China; 2) Genaco Biomedical Products, Inc. USA.

China's family planning program is undertaking a major change. After about 20 years of strict population control, the government is now beginning to focus on promoting prenatal genetic services. There are over 20 million newborns a year in China, however, virtually no prenatal screening service is available for such common diseases as Down syndrome. During the past year, we have established the first large-scale prenatal Down screening and diagnostic program in China. ELISA kits were used for quantitatively measuring maternal serum levels of AFP and hCG. A computer program (Chinese version) was used for risk factor calculations. At risk pregnancies (about 5%) underwent amniocentesis and diagnoses were made by both cytogenetic and molecular genetic analyses. Here, we would like to discuss a strategic issue, i.e., in China, should we change the cut-off value and let more patients undergo amniocentesis to improve Down's detection rate? Currently, patients with risk factors over 1/300 are invited for amniocentesis. Using this cut-off value, about 5% pregnancies will be recognized as "at risk". If all at risk pregnancies elected amniocentesis, about 65% of Down's could be detected. If the cut-off value is changed to 1/500, about 12% pregnancies may require amniocentesis and more than 80% of Down's can be detected. We propose to change the cut-off value based on the following considerations: (1) China still has the "one baby per couple" family planning policy in place. Under this circumstance, patients are more concerned about the "quality" of the baby and therefore, the perceived risk for amniocentesis associated miscarriages is lower. (2) A simple, fast, and cheaper molecular method is used for Down's diagnosis. This method made the prenatal diagnosis more affordable and less labor intensive. Although different social and economical backgrounds may present arguments for making changes in screening strategies, more careful and scientific studies are needed to support such modifications.

Program Nr: 1192 from the 1999 ASHG Annual Meeting

Screening of single nucleotide sequence changes using hairpin-shaped and fluorescently labeled probes. *X. Hu, L. Chen, S. Soares, J. Sorge.* Stratagene Inc, La Jolla, CA.

Single nucleotide sequence changes are the largest source of human DNA sequence diversity and contribute to the development of multifactorial as well as monogenic diseases. The demand is growing for high-throughput methodologies that are sensitive enough to distinguish nucleic acid sequences differing by as few as a single nucleotide. Molecular beacons are hairpin-shaped oligonucleotide probes that fluoresce when hybridized to their target. The presence of the hairpin stem significantly enhances the specificity of the probes, enabling them to distinguish targets that differ by a few down to a single nucleotide. We have developed a series of molecular beacons for the detection of single or multiple nucleotide changes in human genes. These nucleotide changes are associated with disease predisposition or development such as those occurring in the CCR2, CCR5, SDF-1, Factor V, prothrombin, HFE, MTHFR and CFTR genes or with abnormal drug metabolism in the CYP2C19 and NAT2 genes. For the detection of a sequence variation, two molecular beacons with complete sequence match to either the wild-type or the mutant variant are used in the same PCR reaction. Because these molecular beacons are labeled with fluorophores that emit distinct fluorescent light, the three possible allelic representations of a sequence change can be determined simultaneously. In addition, because the test is performed in closed tubes and no post-PCR manipulation of samples is required, the technique saves time and effort and reduces the risk of contamination. Thus, the hairpin-shaped fluorescently labeled probes are particular suitable for high-throughput screening of single nucleotide sequence changes.

Program Nr: 1193 from the 1999 ASHG Annual Meeting

Outcome of the Routine Assessment of Patients with Mental Retardation in a Genetics Clinic. *A. Hunter.* Genetics Program, Children's Hosp Eastern Ont, Ottawa, ON, Canada.

An impression that we had limited success diagnosing children with developmental delay/mental retardation (MR) led us to review hospital and genetics clinic records of 411 index patients seen from 1986 to 1997. Objectives were to establish how often and under what circumstances we made a syndrome/genetic diagnosis and to determine the utility of laboratory tests. This clinic based study differs from series studying institutionalized patients, small groups of children with selected presentation, and clinics specializing in assessment of MR. A specific syndrome/genetic diagnosis was made in 19.9% of cases and in 4.4% the referring diagnosis was excluded. Associated with making a diagnosis was referral from a pediatrician or neurologist, absence of cerebral palsy, the presence of more than 3 minor anomalies and/or an unusual appearance, a recognizable gestalt or a key anomaly. There was a linear relationship between making a diagnosis and the number of minor anomalies. Not associated with making a diagnosis were, year the patient was seen, degree of MR, number of prior specialists, presence of a major malformation, seizures, and head circumference <3rd or >97th%. While chromosome studies were less often ordered in patients with mild MR, the positivity rate was unaffected by severity of MR. It was correlated with the presence of minor anomalies and/or an unusual appearance. None of 134 cytogenetic studies on patients with <=3 minor anomalies alone was diagnostic. Fragile X studies were less often ordered as severity of MR increased; 10/14 positive tests were among those with mild delay; 13/14 diagnoses were predicted on facial gestalt, the 14th had megalotestes and joint laxity. Targeted molecular and FISH studies were limited in number. When ordered by genetics the positive rate was >60%, contrasting 0% when ordered by other physicians. Our use of the laboratory was inconsistent and not clearly based on the findings in a particular child. Changes in referral patterns and the evaluation process could provide significant economies of time and laboratory use, while resulting in few missed diagnoses.

Program Nr: 1194 from the 1999 ASHG Annual Meeting

A rapid and accurate detection of the SMA gene carriers using real-time quantitative PCR. *S. Ichikawa[1], X. Chen[3], P.-Y. Kwok[3], C.H. Wang[1,2].* 1) Department of Biochemistry, University of Missouri-Columbia, Columbia, MO; 2) Department of Psychiatry and Neurology, University of Missouri-Columbia, Columbia, MO; 3) Division of Dermatology, Washington University, St. Louis, MO.

The spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder characterized by degeneration of the spinal cord motor neurons. The gene responsible for SMA has been identified and named survival of motor neuron (SMN). The SMN gene is present in two highly homologous isoforms: the telomeric SMN1 and the centromeric SMN2. These SMN gene copies can be differentiated by a single base-pair polymorphism on exon 7. Currently, the clinical application of this gene discovery has been hampered by the complex genomic organization of the SMN gene copies. The previously reported methods for SMN gene copy number measurement are laborious and sometimes require radioactive labeling. We report here a rapid and accurate method for measurement of SMN1 gene copy number. We developed two allele-specific primers (utilizing the exon 7 polymorphism) to selectively amplify the SMN1 gene copies. We then combined this allele-specific PCR with a TaqMan chemistry to measure SMN1 gene copy number. The ABI PRISM 7700 Sequence Detector allows rapid measurement of SMN1 gene copy number using real-time quantitative PCR. We performed double blind assays to predict the affected and carrier status on 48 individuals from nine SMA families including the probands and the immediate relatives. We predicted that the DNA samples from the SMA patients would retain no SMN1 copy, the carriers would retain one SMN1 copy, and the non-carriers would retain two SMN1 copies. We detected 16 of 17 (94%) affected individuals, 25 of 25 (100%) carriers, and 5 of 6 (83%) non-carriers. The overall accuracy was 96% (46 of 48). The affected status was confirmed by SMN1 gene deletion assays using SSCP, and the carrier status was confirmed by microsatellite genotyping using markers flanking the SMN gene on chromosome 5q13. These results indicate that this new method combining allele-specific PCR and TaqMan chemistry can be used for a rapid and accurate detection of the SMA mutant gene carriers.

Program Nr: 1195 from the 1999 ASHG Annual Meeting

Delivery of genetic services to the Manitoba Hutterites in the molecular era. *A.M. Innes[1], K. Wrogemann[1], T. Zelinski[1], G. Coghlan[1], S. Maendel[2], M. Maendel[2], J. Evans[1], C.R. Greenberg[1]*. 1) Department of Biochemistry and Medical Genetics, University of Manitoba, Winnipeg, MB, Canada; 2) Fairholm Hutterite Colony, Portage La Prairie, MB, Canada.

The Hutterites are an Anabaptist sect living on the North American prairies, including the Canadian province of Manitoba. They have a number of features relevant to studies of genetic diseases including high rates of fertility, high levels of consanguinity and excellent genealogic records. In addition, virtually all Hutterites descend from fewer than 60 common founders. A comprehensive list of genetic disorders in the Hutterites has been published only once previously (Bowen,P. Am J Med Genet. 22:449-51, 1985). We report herein an updated list of disorders observed in the Hutterites obtained by reviewing the database of the Section of Genetics and Metabolism at the University of Manitoba. More than 25 Mendelian disorders and traits have been identified amongst the Hutterites including those unique to this population (Bowen-Conradi syndrome, Wd blood group), and disorders over-represented in this population (carnitine palmitoyl transferase-1 deficiency, Limb-Girdle Muscular Dystrophy) as well as more common diseases (Cystic Fibrosis). The genes for many of these disorders have now been cloned and, in some instances, specific Hutterite mutations have been characterized. Work is ongoing in our laboratories to map and clone other disease-associated genes found in this population. In the past, we have found individual Hutterite families to be receptive to and highly interested in genetic counseling. Given the progress at the molecular level, and given the importance of community autonomy and participation with respect to genetic health issues, we have initiated discussions with representatives of the community as a whole. We hope to provide the Hutterites with a more comprehensive appreciation of their genetic heritage and to present options including community based screening for specific disorders. Preliminary discussions suggest a high level of interest in pursuing novel approaches to delivery of education and genetic testing to this community.

Program Nr: 1196 from the 1999 ASHG Annual Meeting

DHPLC analysis of all coding exons of TSC1 and TSC2: a highly sensitive screen for molecular diagnosis. *A.C. Jones[1], B. Hoogendoorn[2], M.C. O'Donovan[2], J.R. Sampson[1], J.P. Cheadle[1]*. 1) Institute of Medical Genetics, UWCM, Cardiff, Wales, UK; 2) Department of Psychological Medicine, UWCM, Cardiff, Wales, UK.

The TSC1 and TSC2 genes are mutated in tuberous sclerosis. They have complex genomic structures (21 and 41 coding exons) and exhibit diverse mutational spectra with few recurrent mutations. We have developed a rapid semi-automated screen of all TSC1 and TSC2 coding exons and splice junctions using denaturing high performance liquid chromatography. Analysis was performed at the melt temperature suggested by the DHPLCMelt software (RTm) and RTm + 2 °C. Sensitivity in a series of 150 unrelated TSC families is in excess of 66% (99 mutations identified with analysis at this time incomplete in samples from 27 cases). This compares favourably with SSCP (sensitivity 61%) and heteroduplex analyses (58%) which have already been completed for all patients. Using Southern blot analysis and long PCR 24 of the 150 cases (16%) were found to have whole exon or multi-exon deletions that can not be detected by any of the exon screening approaches.

Program Nr: 1197 from the 1999 ASHG Annual Meeting

Pharmacogenomic study outcome models for quantitative drug-response traits. *D.A. Katz[1], F. Yang[1], N.J. Schork[2].* 1) Abbott Laboratories, Abbott Park, IL; 2) Genset Corporation, La Jolla, CA.

When undertaking a pharmacogenomic association study, prior assessment of the likelihood of various genetic hypotheses is useful. Favored genetic hypotheses can be used as models to assess the potential utility of the study. An observed trait distribution can be thought of as the sum of several component distributions. A statistical test is applied to determine whether a sum of components explains the observed data better than a single skewed component. Each component distribution could represent the distribution of trait values for the population having a certain gene. For an available set of patient samples, the power to detect the genes represented in each model can be calculated. By setting clinically relevant threshholds, the sensitivity and specificity of a diagnostic test for each gene can be determined. We will demonstrate these procedures utilizing serum alanine aminotransferase data from a long-term study of the leukotriene synthesis inhibitor Zyflo®, and the sample sets now in use to discover genes related to drug-related hepatic adverse events. We will show how characteristics of the genetic map used to find genes, sample size, and the genes themselves influence study power.

Program Nr: 1198 from the 1999 ASHG Annual Meeting

Detection of cryptic Y chromosome mosaicism by coamplification PCR with archived cytogenetic slides of Turner syndrome patients. *J.W. Kim[1], E.H. Cho[1], Y.M. Kim[1], J.Y. Han[2], J.Y. Jun[2], S.Y. Park[1]*. 1) Genetic Research Laboratory, Samsung Cheil Hospital, Seoul, Korea; 2) Obstetrics and Gynecology, Samsung Cheil Hospital, Seoul, Korea.

Turner syndrome is one of the most common cytogenetic abnormality. Y chromosome or Y-derived material is present in 6-9% Turner syndrome patients. It is known that the presence of Y chromosome may predispose them to gonadoblatoma formation with an estimate risk of 15-25%, so it is crucial to identify whether Turner syndorme patients have Y-derived material. In this study, 26 archived slides, which were analyzed as pure 45,X and mosaic 45,X/46,XX by cytogenetic analysis, were screened. Coamplification PCR having the advantage of providing rapid results and confirming PCR failure was performed using the exon 46 of dystrophin gene in Xp21 and DYZ3 of Y centromeric region. One archived slide of pure 45,X cytogenetically was found to have cryptic Y-derived materials. The result of this study indicates that the archived cytogenetic slides can be rapidly and efficiently applied for the detection of hidden Y chromosome in Turner syndrome patients.

Program Nr: 1199 from the 1999 ASHG Annual Meeting

Cancer risk assessment programs--whose domain? *E.R. Knell, C. Presant.* Los Angeles Oncologic Institute, Los Angeles, CA.

Cancer risk assessment, once primarily limited to the research realm, has moved into clinical practice, but little research has been done to analyze and compare aspects of these programs. We surveyed our genetic counseling colleagues to ascertain how their programs are coordinated and conducted, sampling from counselors throughout the country, to enable an objective comparison of some aspects of these programs. This survey did not include programs conducted only by physicians, as sampling was through the NSGC cancer risk assessment interest group.

Of the 30 institutions responding, 20 were not for profit, 7 for profit, and 3 did not state. Overwhelmingly, they were clinical (N=19, 63%), or a combination of both research and clinical (N=9, 30%). While the majority (N=17, 57%) relied on a team of the genetic counselor and oncologist to determine if and which genetic testing would be offered, the genetic counselor was the sole consultant in 11 (36.7%) of programs. Most programs offered 2-4 sessions, and often (N=22, 73%) the genetic counselor was the only professional for the first one or several sessions, encompassing genetic education and often risk assessment. For the result sessions, if testing was done, an oncologist and genetic counselor were usually present (N=21, 70%), although often the physician was not in attendance for the entire session. This presentation will include a review of these and other aspects of these programs, stressing the importance of a team approach but acknowledging the often primary role of the genetic counselor in many programs. This key role for the genetic counselor is a corollary of the extensive time requirement of counseling services and the technical and specialized knowledge required of the assessment and other phases of the consultation.

Program Nr: 1200 from the 1999 ASHG Annual Meeting

Trisomy 9 mosaicism detected by interphase FISH in noninvasively obtained and directly prepared tissues. *J. Kobori, B. Pettersen, K. Nguyen, S. Lew, V. Marquez, X. Li.* Genetics Department, Kaiser Permanente Northern California, San Jose, CA.

Mosaic chromosome aneuploidy can have variable presentations and phenotypes. Suspicion of a mosaic chromosome aneuploidy when lymphocyte analysis reveals a normal karyotype generally requires analysis of invasively obtained tissue such as skin fibroblast culture. To study the possibility of mosaic trisomy 9 in a 15 year old boy with severe mental retardation, aggressive behavior, and dysmorphic features, cells were collected noninvasively from buccal smear and urine, and directly prepared for fluorescence in situ hybridization (FISH) analysis. F.V. was born full term in the Philippines after an uncomplicated pregnancy, labor, and delivery. Cytogenetic analysis of blood lymphocytes was done at one year of age to evaluate dysmorphic features and developmental delay. Results were reported as trisomy 9. Original data from this study are not available. Genetics evaluation in the U.S. at age thirteen years revealed normal physical growth. He had dysmorphic features including tall forehead, flat occiput with low posterior hair line, arched and medial flare to eyebrows, deep-set upslanting eyes with anterior displacement of inner canthi, simple ears, short and broad nose, high narrow palate, and joint hyperextensibility with short distal phalanges. Repeat cytogenetic analysis of blood lymphocytes showed a normal male karyotype in 100 cells. Interphase FISH using dual-color labeled ABL/BCR DNA probes (Vysis, Inc.) specific for 9q34 and 22q11.2 (as an internal control) revealed trisomy 9 in 3% (3/100) of cells in buccal smear and 12% (12/100) in urine. FISH analysis of a normal control using the same probes showed no cells with trisomy 9 (0/100). This result confirms the diagnosis of trisomy 9 mosaicism. This patient's limited phenotype can be explained by the variable distribution of trisomy 9 mosaicism. This technique is a simple, noninvasive, and reliable tool for the study of chromosome aneuploid mosaicism. Its application should be considered prior to analysis of invasively collected tissues.

Program Nr: 1201 from the 1999 ASHG Annual Meeting

Detection of collagen I, II and XI gene defects in osteochondrodysplasias. *J. Korkko, L. Ala-Kokko, M. Kinnarney, D. Prockop.* Ctr Gene Therapy, MCP Hahnemann Univ, Philadelphia, PA.

Collagen I is the major protein of skin, tendon and bone; collagen II is one of the main components of cartilage and vitreous of the eye. Collagen XI is a minor cartilage component co-expressed with collagen II. The genes coding for these proteins are complex in that they consist of over 50 exons and are repetitive. Therefore it's been difficult to establish a practical and reliable method for mutations detection. We have adapted conformation sensitive gel electrophoresis (CSGE)procedure to analyze these genes for mutations in osteochondrodysplasia patients. The mutation detection rates were the following:

Gene(s) analyzed	Referral diagnosis	Found/studied	%
COL1A1/COL1A2	OI I	49/56	88%
	OI II and III	34/41	83%
	OI IV	11/15	73%
	OI vs child abuse	2/25	8%
	Osteoporosis	1/52	2%
COL2A1	Achondrogenesis II	19/20	95%
	SED, SEMD	21/33	64%
COL2A1/COL11A1	Stickler/Marshall sd	43/58	74%

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Program Nr: 1202 from the 1999 ASHG Annual Meeting

Genetic and Health Care Decisions of Adolescent Daughters of Breast Cancer Patients. *Y.G. Korneluk[1], M. Cappelli[1], S. Verma[2], A. Hunter[1], E. Tomiak[2], J. Allanson[1], C. DeGrasse[3], L. Corsini[2].* 1) Children's Hospital of Eastern Ontario Ottawa, Ontario, Canada; 2) Ottawa Regional Cancer Centre; 3) Ottawa Regional Women's Breast Health Centre.

Adolescent daughters of breast cancer patients are often viewed by oncologists and other health care professionals as “the patients of the future” because some are at increased risk for the development of breast cancer. Furthermore, compared to other family members, they may also be at increased risk for emotional problems when their mothers are diagnosed with the disease. In light of the high incidence of breast cancer and recent developments breast cancer genetics, it is important that we develop effective ways of educating and treating these patients of the future. Our primary goals are to investiage: (a) the adolescent's thoughts and plans with respect to their own breast health, including attitudes towards genetic testing, (b) the psychological functioning of adolescent girls at risk for a hereditary form of breast cancer, and (c) family coping with the illness. We are utilizing a case-comparison design to compare 100 families in which the mother has been diagnosed with breast cancer (BC) to 100 families from the general population (GP) on a variety of self-report measures and an interview. Our protocol was established with the help of focus groups conducted with families with adolescent daughters (N= 16). Data collection has begun, and we have recruited 53 families (N =41 GP and N = 12 BC) to date. We predict that factors related to family coping and attitudes about breast cancer prevention and treatment will be associated with adolescent daughter's functioning and attitudes about breast cancer treatment, prevention, and detection strategies including breast cancer gene testing.

Program Nr: 1203 from the 1999 ASHG Annual Meeting

Diagnosis of maternal UPD 7 by methylation specific PCR. *R. Kosaki[1,2], K. Kosaki[2,3], W. Robinson[4], L. Shaffer[5], W. Craigen[5], N. Matsuo[1].* 1) Health Ctr, Keio Univ; 2) Dept of Peds; 3) Pharmacia-Upjohn Fund for Growth & Development Research, Keio Univ Sch of Med, Tokyo, Japan; 4) Dept of Med Genet, Univ of British Columbia, Vancouver, Canada; 5) Dept of Mol Hum Genet, Baylor Col of Med, Houston, TX.

About 10 percent of patients with Russell-Silver syndrome have maternal uniparental disomy of chromosome 7 [UPD7]. The purpose of this report is to present a methylation specific PCR assay [MSPCR] to diagnose maternal UPD7 by analyzing methylation status the PEG1/MEST, the only imprinted gene on chromosome 7. The putative promoter of the PEG1 contains a CpG island which is methylated on the maternal allele and unmethylated on the paternal allele. Therefore, in patients with maternal UPD7, only the methylated allele is present. In order to evaluate methylation status of PEG1 gene, methylation specific PCR was used. From the published genomic sequence of the putative promoter region of the PEG1 gene (GenBank #Y10620), DNA sequences after bisulfite modification were deduced by converting all cytosines to thymines for the unmethylated allele and by converting all cytosines to thymines with the exception of those in the CpG dinucleotide for the methylated allele. Based on the differences between two sequences, methylated allele- and unmethylated allele- specific primer pairs were designed. Bisulfite-modified DNA from 4 patients with maternal UPD7 amplified only with the methylated allele specific- pair while modified DNA from a patients with paternal UPD7 amplified only with the unmethylated allele-specific pair. Modified DNA from 50 normal unrelated individuals amplified with both primer pairs. The advantages of the present method over conventional linkage analysis is as follows: 1) MSPCR can be performed without obtaining parents samples. In this regard, MSPCR is not subject to the problem of non-paternity, which jeopardize the result of linkage testing; 2) MSPCR consists of only two sets of PCR while linkage analysis requires evaluation of multiple markers because informativeness of the markers is not known prior to testing. We conclude that MSPCR assay is a simple and robust assay for maternal UPD7.

Program Nr: 1204 from the 1999 ASHG Annual Meeting

Client-defined outcomes for clinical genetic services. *S.T. Kozel[1], H. Radtke[1], C. Mills-Lovell[1], C. Ferrang[2], D.B. Flannery[1]*. 1) Department of Pediatrics, Medical College of Georgia, Augusta, GA; 2) Quality Management Program, Medical College of Georgia.

Quality of health care services is assessed through measurement of outcomes, yet defining valid outcomes for clinical genetic services has been an elusive task. A continuous quality improvement program at the Medical College of Georgia afforded the opportunity to discover patient/families' perspectives in identifying outcomes of genetic services. Each patient/family ("client") received a satisfaction survey to complete anonymously at the end of their clinic visit. One of the questions in the survey asked if the clients found the genetic visit helpful; another, open-ended, question asked the clients to identify what aspects of the service were helpful. 444 surveys were distributed; 237 were returned (53.4%). 226 responded to the helpfulness question; 225 reported the service was helpful (99.6%). 158 clients responded to the open-ended question; many wrote multiple responses. Responses to this question were analyzed, and 5 themes were identified. 72.1% reported that **gaining knowledge and understanding** was a helpful result of the service. Examples cited included reaching a diagnosis, and understanding the implications of the condition. A **decrease in worry and anxiety** was a helpful outcome for 11.6%. For example, clients reported relief when told that their child's development was proceeding as expected. An **increased sense of personal control** was reported by 9.3%, including being able to use information gained to determine whether or not to have another child. A **decreased sense of guilt** was identified by 5.8% as a helpful outcome of the service, as a result of understanding the cause of the condition. 12.8% of the responses could only be placed in an "**other**" category. Most of these responses stated that "everything" was helpful, or that the care and concern of the genetics team was helpful. It can be concluded that almost all clients perceived genetic services to be beneficial. These data suggest that future studies should focus on the above five themes for the development of outcome measures to assess the quality of genetic services.

Program Nr: 1205 from the 1999 ASHG Annual Meeting

Genetic research on anonymous samples and data - benefits exceeding impediments. *K.T. Kristjansson, R.Th. Valdimarsson, J. Gulcher, H. Gudbjartsson, K. Stefansson.* deCODE Genetics, Inc, Reykjavik, Iceland.

Modern computer technology allows for extensive data collection and handling of information like never before, making issues about privacy of personal information more valid than ever. In the field of medical research there are increased demands for irreversibly removing all personal identifiers off samples and data. This can have devastating effect on the research value of collected genetic material and data banks. On the other hand, the patients and participants in our research deserve the best possible protection but effective usage of their personal information and biological samples they have donated for the purpose of scientific advancements in medicine for current and future generations. We have developed a comprehensive custom-made computer system that stores and maintains anonymous information about individuals in research projects. It keeps track of blood and DNA samples, phenotypes and the genealogy at every stage from the time that the blood enters the lab till the creation of genotypes. Personal identifiers are encrypted using an 128-bit symmetric encryption algorithm (Advanced Encryption Standard Candidate). The encryption methods are complemented with access restrictions, making the system very secure. A governmental official who stores the encryption key secures anonymity of the samples and the data. The components of the system, except for the coding /decoding, are tied together in a centralized ORACLE database server. They are interactive and accessible through a simple to use graphical interface. This system provides the following benefits to genetic research: a) Privacy to personal information, b) Possibilities of updating information on individuals without risking privacy b) Minimizes losses and erroneous handling by incorrect placement or record keeping by humans, c) Fast genealogy and pedigree drawing, d) Easy data managing and research planning, e) Flexibility in genotype/phenotype correlation, f) Quality control of PCR and genotyping results, g) Minimizing the possibility of result biasing by facilitating blinded genotype analyses.

Program Nr: 1206 from the 1999 ASHG Annual Meeting

Mutation detection using DNA chromatography (DHPLC). *A.I. Kuklin[1], R. Haefele[2], P.D. Taylor[2], D.T. Gjerde[2], K. Hecker[2], K. Munson[1]*. 1) Applications Laboratory, Transgenomic Inc., San Jose, CA; 2) Research and Development, Transgenomic Inc., San Jose, CA.

A novel polymer matrix together with instrumentation and software modeling of the DNA-polymer interaction, now offers an alternative to gel-based analysis of DNA for SNP/mutation discovery and other applications. The technology provides an automated alternative to SSCP and DGGE for mutation scanning with no a priori knowledge of the nature of the location of the mutant. Here we present data and discuss optimization of protocols for pooling so that a higher throughput is achieved in scanning for sequence variants. We demonstrate the applicability of fluorescence detection in mutation screening and discuss various technical approaches.

Program Nr: 1207 from the 1999 ASHG Annual Meeting

The performance of fetal cell sorting by magnetic-activated cell sorting and FISH - a ciritical evaluation. *P. Kuo.* Dept OB/GYN, National Cheng-Kung Univ Hosp, Tainan, Taiwan, Republic of China.

Background: Although advances have been made in the enrichment and isolation of fetal cells for analysis, there is still no consensus on the frequency of fetal NRBCs in maternal blood. The inconsistent findings of the previous studies most likely arise from the highly variable performance of the current sorting technologies and the inadequate enumeration of fetal cells. This prompted us to investigate the perofrmance of magnetic-activated cell sorting (MACS) and FISH in a systemic approach. Materials and Methods: From each of the 43 participants, 30-40 ml of peripheral venous blood was collected at 10-16 weeks' gestation. Approximately 3x10,000,000 nucleated cells were subjected to the initial separation using a triple density gradient of Histopaque. After the initial separation, the cells were equally subdivided into three parts. Two parts were simultaneously subjected to two separate miniMAC columns for selection of CD 71-positive cells. The remaining cells were subjected to amplification of the ZFY gene by nested PCR. All cells in the CD 71-positive fraction were evaluated with dual-color FISH to detect XY cells. Results: The number of cells in the CD 71-positive fraction ranged from 43000 to 926000. Although there is no statistically significant difference between the results obtained from two columns, the results were highly variable for some paired experiments. In 22 pregnancies carrying the male fetus, the number of XY cells ranged from 0-76. The estimated frequencies of XY cells in the CD 71-positive fractions were also highly variable for some paired experiments. The sensitivity of detecting XY cells was 95.5% (42/44) for FISH and 100% (22/22) for PCR. In pregnancies carrying the female fetus, XY cells could be detected by both FISH and PCR in two, and by PCR only in one. The number of XY cells ranged from 1 to 11. Discussion: The present study clearly demonstrates the highly variable performance of MACS in fetal cell isolation. Detection of male cells in pregnancies carrying the female fetus may reflect vanishing-twin phenomenon or contamination by granulocytes/lymphocytes persisting from previous male pregnancies.

Program Nr: 1208 from the 1999 ASHG Annual Meeting

Arrayed Primer EXtension (APEX) as a new DNA resequencing and mutation detection technology. *A. Kurg[1], N. Tõnisson[1], E. Lõhmussaar[1], A. Metspalu[1,2].* 1) Institute of Molecular and Cell Biology, University of Tartu, Estonian Biocentre, Tartu, Estonia; 2) Asper Ltd., Tartu, Estonia.

Development of a method for rapid identification of large number of mutations is an attractive challenge for medical genetics. A current alternative to gel-based methods is the use of DNA microarrays, devices displaying specific oligonucleotides or DNA fragments, precisely located on a small-format solid support. We present Arrayed Primer EXtension (APEX) method that combines the advantages of both, an array-based method, with that of the Sanger dye terminator method. We describe an integrated system with chip and template preparation, multiplex primer extension on the array, fluorescence imaging equipment and data analysis. The method is based upon a two dimensional array of oligonucleotides, immobilized via 5' end on a glass surface. A patient DNA sample is amplified by PCR, digested enzymatically, and then annealed to the immobilized primers, which promote sites for template-dependent DNA polymerase extension reactions. Four unique fluorescently labeled dideoxy nucleotides are used to extend each primer by only one base. A mutation will be detected by a change in the color code of the primer sites. We have developed and realized a total internal reflection fluorescence (TIRF) based excitation mechanism combined with a CCD camera detector for high-throughput image acquisition. The signals from the spectrally separated dyes are resolved with dual selection by excitation with color-specific laser and band-bass filtering of the emitted fluorescence. Imaging is followed by a software analysis to convert the fluorescence information into sequence data. The signal to noise ratio of APEX is, on the average 30:1 which enables identification of a heterozygous mutation with a high confidence level. The systems currently used include resequencing of the p53, -thalassemia, and BRCA1/BRCA2 mutation detection tests and a system to analyze genome-wide SNP markers.

Program Nr: 1209 from the 1999 ASHG Annual Meeting

Minisatellite Analysis Clarifies Mutation, Nonidentity and Sample History. *R.V. Lebo, T. Maher, L. Farrer, E. Yosunkaya, J. Milunsky.* Center for Human Genetics and Depts. Pediatrics and Medicine, Boston University School Medicine, Boston, MA.

Highly polymorphic simple sequence repeats (SSRs) provide a ready resource to resolve (1) meiotic repeat number modification or (2) failure to PCR amplify an allelic site from (3) nonparentage (4) maternal cell contamination of a fetal sample, (5) abnormal PCR amplification, (6) twin or sample nonidentity and (7) questionable sample history. These categories have been resolved routinely with a posterior likelihood of the correct categorization exceeding 99.9 percent. This reliability, which is required to introduce most DNA evidence into a court of law, provides a robust standard to resolve molecular diagnostic ambiguities. Among the 48 pedigrees tested for spinal muscular atrophy linkage and 155 tested to determine parentage, we characterized 4 cases in which an offspring did not carry a parental allele resulting from failure to amplify one allelic target site or meiotic repeat number modification. Standard parentage testing with both parents is routinely completed using six unlinked SSR loci with calculated heterozygosities exceeding .80. Testing twelve loci readily resolved parentage with only one available parent. In the 4 cases that a parent failed to pass one allele to his offspring at one tested locus, parentage was resolved by testing six additional unlinked loci and three to six syntenic loci. Removal of artifactual PCR amplification bands was accomplished by further purification or dilution of DNA samples. Suspected maternal cell contamination detected by simple sequence repeat (SSR) analysis of DNA extracted from a CVS cell culture was confirmed by amniocentesis. The CVS culture gave a normal genotypic result at the SMA locus while the follow-up amniocentesis found the fetus was affected with SMA and differed with the mother's SSR alleles at multiple loci. In summary, while complex results were obtained initially in 4 in 203 pedigrees and 6 of 9,000 individual DNA samples, testing highly polymorphic SSR loci readily distinguished background inherited variability from technical artifact, questionable parentage and questionable sample history.

Program Nr: 1210 from the 1999 ASHG Annual Meeting

Rapid detection of a-**thalassaemia deletions and** a-**globin gene triplications by multiplex PCRs.** *Y.T. Liu[1], K. Miles[1], J.M. Old[2], C.A. Fisher[2], D.J. Weatherall[1], J.B. Clegg[1].* 1) MRC Molecular Haematology Unit, Institute of Molecular Medicine, University of Oxford; 2) National Haemoglobinopathy Reference Service, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, UK.

The a-thalassaemias are the most common single-gene diseases in the world. They are characterized by a reduction or complete absence of a-globin gene expression. Normal individuals have two a genes on each chromosome 16 (aa/aa). The loss of one (-a) or both (--) of these *cis*-linked genes is the most common cause of a-thalassaemia. Traditionally, molecular characterization of a-thalassaemias has been carried out using Southern blot analysis. The method is both labour-intensive and expensive. Moreover, its success depends heavily on the quality of the genomic DNA under test and the quantity available. PCR-based assays, which are more rapid, less expensive and more sensitive, have been described previously, but for various reasons have not found their way into routine use. Since the majority of a-thalassaemias worldwide are mainly caused by a few common underlying deletions, e.g. $-a^{3.7}$, $-a^{4.2}$, $--^{SEA}$, $--^{MED}$, $-(a)^{20.5}$, $--^{FIL}$ and $--^{THAI}$, we developed a universal PCR approach, based on three simple and reliable multiplex PCR assays, that allows rapid detection of these seven common deletions as well as the $aaa^{anti3.7}$ and $aaa^{anti4.2}$ triplications, which could be useful in predicting the clinical phenotype of thalassaemia intermedia. Furthermore, in populations where b-thalassaemia with a normal HbA_2 is common, a simple and reliable method for distinguishing heterozygous a^0-thalassaemia from b-thalassaemia trait would also facilitate carrier screening and prenatal diagnosis for b-thalassaemia. We are applying this strategy to screen population samples for these common a-thalassaemia deletions from Southeast Asians to determine their population frequencies. Additional applications, including multiplex amplification from whole blood, will be presented.

Program Nr: 1211 from the 1999 ASHG Annual Meeting

Psycho-social and familial impact of liver transplantation in patients with Familial Amyloid Polyneuropathy (FAP). *A. Lopes[1,2], M. Branco[1], M. Fleming[1], T. Coelho[1], A. Sousa[1], M. Caetano-Pereira[2]*. 1) Centro Estudos Paramiloidose, Porto, Portugal; 2) Transplantation Department, HGSA, Porto, Portugal.

Liver transplantation (L. T.) changed considerably the way FAP patients face and live their diseases. At the same time it imposes an important psychological burden at the moment of the transplantation. In order to evaluate and understand these issues a study of psychological, familial and social impact was designed, whose aim was the assessment of the following dimensions: *Psychopathological traits, Depression, Anxiety and Quality of Life*.

Thirty-one patients transplanted over one year (1997-1998) were evaluated using the following instruments: *Quality of Life Scale (SF 36), Depresssion Inventory (IACLIDE), Brief Symptom Inventory (BSI), Rorschach Test and a Half Structured Interview*. Two moments of evaluation were chosen: just before liver transplantation and nine to twelve months after. Another group of FAP patients refused for surgery was evaluated with same instruments.

Changes in depression, anxiety, somatization and some dimensions of quality of life were found. After L.T. the subjets presented: (1) lower levels of somatization, depression and anxiety (measured in BSI); (2) lower levels of depression (measured in IACLIDE); (3) upper levels of physical functioning, general health, vitality and social functioning (measured in SF36).

These results indicate that L.T. has an important impact on psychic and social life of FAP patients. They also point out to the need of psychological support during that period.

Program Nr: 1212 from the 1999 ASHG Annual Meeting

Direct comparison of SAGE and GeneChip on quantitative accuracy in the transcript profiling analysis. *H. Aburatani[1], M. Ishii[1], S. Hashimoto[2], S. Tsutsumi[1], Y. Wada[1], K. Matsushima[2], T. Kodama[1].* 1) RCAST, Univ Tokyo, Tokyo, Japan; 2) Dept Mol Prevent Med, Univ Tokyo, Tokyo, Japan.

Among high throughput, comprehensive analysis of relative transcript expression levels, Serial Analysis of Gene Expression (SAGE) and oligonucleotide array based hybridization are currently common approaches. To compare quantitative accuracy of those methods, both analysis were carried out using the identical RNA specimens, which were prepared from blood monocytes and GM-CSF induced macrophages to study the transcript profiling in the process of differentiation from human blood monocytes into macrophages. By SAGE analysis 57,560 and 57,463 tags were obtained from monocytes and macrophages, respectively, resulting in nearly 28,000 different tags, while the oligo array hybridization was performed with Genechip (Affymetrix). As expected, the orders of mRNA abundance in each sample were not necessarily very well correlated between the two methods, probably because the hybridization intensity depends on the sequences of oligonucleotides on the array. However, comparative changes during the differentiation were very similar. Out of 20 genes, which showed highest folds of increase in expression levels, twelve were shared between the both analyses, i.e. apoE, type IV collagenase, GP-39, apoC-I, etc. These suggested that Genechip technology is reliable enough in quantitative analysis of expression profiling, although genes not present on the array cannot be analysed. Disadvantages with SAGE would be that occasionally no definite gene was assigned for a tag, or even no match found in the database. Discrepancy of the expression level was found in several genes, which are currently investigated.

Program Nr: 1213 from the 1999 ASHG Annual Meeting

Construction of 6 Mb bacterial clone contig in the reading disability region on 6p22. *J. Ahn, A. Zia, T. Won, J.R. Gruen.* Pediatrics, Yale School of Medicine, New Haven, CT.

Four independent mapping studies by 3 different groups consistently identifies a locus for developmental reading disability on 6p21.3-22. Locus size within this broad region ranges from 11cM spanning D6S422 (pter) through D6S291 (Fisher et al., AJHG, 64:146, 1999), and 10cM spanning D6S109 through D6S306 (Grigorenko et al., AJHG, 60:27, 1997), to 5cM spanning D6S461 through D6S258 (Gayan et al., AJHG, 64:157, 1999). While the centromeric portions of the common overlapping regions have been cloned in sequence-ready BAC and PAC contigs, the middle and telomeric portions are covered only by YACs. In order to complete the cloning of this region we assembled a bacterial clone contig consisting of PACs and BACs spanning approximately 6 Mb on 6p22, and markers pter-D6S1950-D6S1621-cen. Human insert DNA of YACs 966E10, 958H9, 935A8, 901A10, and 947F6, were amplified by inter-IRE and IRE-bubble PCR methods and used to screen the RPCI-11 human male BAC and the RPCI-1 human PAC libraries (Pieter de Jong, Roswell Park Cancer Institute, http://bacpac.med.buffalo.edu). The resulting BACs and PACs were grouped into bins using 74 unique STS, STR, and EST markers that were previously mapped to corresponding YACs. The bins were then connected to form a contig of approximately 6 Mb using additional STSs developed from cloned end sequences. We grouped 182 BACs and 15 PACs into 3 unconnected bins. We estimate that the bins cover approximately 5.5 Mb of genomic DNA based on the number and the sizes of clone inserts. This contig will be useful for ordering and for isolating more genetic markers, and to narrow down the reading disability locus further.

Program Nr: 1214 from the 1999 ASHG Annual Meeting

Rapid identification of polymorphisms in genomic DNA using long range PCR and transposon based sequencing.
A. Antonellis, S.C. Curtis, M.G. Pezzolesi, D.K. Moczulski, A.S. Krolewski. Joslin Diabetes Center, Harvard Medical School, Boston, MA.

As the human genome sequencing project nears completion it is becoming very important to obtain panels of polymorphisms and to construct these into physical haplotypes. To remain competitive, it will be necessary to screen large amounts of genomic DNA rapidly, accurately and efficiently. The current methods of detection are limited by the need to design and generate many products for analysis. This can delay the detection of polymorphisms by weeks or months; even more if the region has not yet been sequenced. To expedite this process we have developed a technique to screen 10kb fragments of known or unknown genomic DNA using a combination of long range PCR and transposon based sequencing.

Using DNA from 4 individuals we designed long range PCR for all 3 introns and 10kb flanking regions of the type 1 angiotensin II receptor (AT1) gene. Once amplified, we subcloned the products from each individual and selected 12 valid clones per person. The DNA samples from these clones were pooled and a transposon insertion reaction was performed. After transforming and plating this reaction, 192 clones were selected and sequenced from both sides of the internal transposon. This provided 16X coverage and the complete sequence of the unknown regions. Polymorphisms were identified as points in the assembled sequence that showed conflicting data and that occurred in more than one reaction. All polymorphisms were confirmed by direct sequencing of PCR products from the four individuals studied. Haplotypes were formed by sequencing individual monochromosomal clones for the presence of all valid polymorphisms. Processing each 10kb fragment took two weeks.

This methodology provided us with a useful map of polymorphisms to begin association studies with. We therefore conclude that this is a very powerful tool for rapidly identifying polymorphisms with a frequency of .20 or greater and for constructing long range, physical haplotypes.

Program Nr: 1215 from the 1999 ASHG Annual Meeting

Physical characterization of distal Xq28 and the syntenic mouse region: candidate gene analysis for Incontinentia Pigmenti Type 2. *S. Aradhya, D.L. Nelson, & the International IP2 Consortium.* Molecular & Human Genetics, Baylor College of Medicine, Houston, TX.

Incontinentia Pigmenti Type 2 (IP2) is a neurocutaneous genodermatosis that is localized in distal Xq28 and segregates as a dominant and male lethal trait. Current physical maps in the critical region are poorly characterized, consequently hindering accurate mapping of markers, gene structure analyses and sequencing. We have constructed a BAC contig between G6PD and the Xq telomere to assist with gene studies for identifying the IP2 mutation. While confirming that clones map to Xq28 by fluorescence in-situ hybridization, we identified a pseudogene whose original copy lies on the 6q telomere. Moreover, clones betweeen DXS115-2 and DXS115-3 revealed paralogous sequences on three other chromosomes. We have also isolated mouse PAC clones to build a contig across the mouse region(s) syntenic with the human IP2 region. Mapping of mouse Mtcp1, Vbp1 and Xap130 genes indicated a break in synteny such that genes between Dxhxs52 and Vbp1 map in the middle of the murine X chromosome while Xap130 maps near the centromere. Also, a pseudogene for Vbp1 exists on mouse chromosome 3. Finally, we have performed mutation screens on 10 genes as possible candidates for IP2 using heteroduplex analysis and Southern blotting. No mutations have been found; however, several single nucleotide polymorphisms were observed.*Mtcp1Vbp1DXS115-2DXS115-3G6PDDxhxs52Xap130in-situ.*

Program Nr: 1216 from the 1999 ASHG Annual Meeting

Identification of polymorphisms affecting gene expression. *J.C Austin, B. Hoogendoorn, T. Bowen, A. Halsall, S.L Coleman, M.C O'Donovan, P. Buckland.* Department of Psychological Medicine, University of Wales College of Medicine, Heath Park, Cardiff, CF4 4XN, Wales, U.K.

The identification of common functional polymorphisms is a key objective for the human genomics community. Funded by the MRC (UK) we are contributing to this objective by 1. Screening the promoter regions of 2000 genes for common polymorphisms using denaturing high performance liquid chromatography (DHPLC) 2.Testing identified polymorphisms for functional consequences in a reporter gene system. Because our primary interest in this initiative is to facilitate the genetic analysis of neuropsychiatric disorders, this programme will focus on genes of interest to research groups working in this field. Work on the first 200 promoters suggest that the first 500 bases in the 5' flanking region of genes is a rich source of polymorphisms which are present in approximately 50% of screened fragments to date. We anticipate generating a valuable resource for the international neuropsychiatric genetics community that will 1. Contain dense maps of polymorphisms for LD studies in regions of interest to neuropsychiatric genetics groups. 2. Contain a database of polymorphisms tagged to approximately 1000 genes for LD studies of positional and functional candidate genes. 3. Contain information on functional polymorphisms regulating the expression of genes for testing in association studies of complex diseases.

Program Nr: 1217 from the 1999 ASHG Annual Meeting

Improvements in resolution and reproducibility of short tandem repeat (STR) analysis on automated slab gel apparatus. *W.R. Baubonis, D.G. MacDougall, M. Wu, T.D. Zhang, J.M. O'Connor, K.S. Seidell, M.M. Garner.* FMC BioProducts, Rockland, ME.

Analysis of Short Tandem Repeats [STRs] is the most widely used method for genotyping and forensic identification of individuals. Although Single Nucleotide Polymorphism [SNP] analysis may eventually prove more facile and rapid, the STR population databases which are already available allow for genotype-phenotype correlations to be drawn or discrete probabilities of identification of an individual to be calculated. STR analysis is commonly carried out on automated instruments, either by slab gel or capillary electrophoresis. The ability to resolve alleles with different numbers of repeats and, for automated assays, the reproducibility of the absolute electrophoretic mobility of each repeat allele, are crucial for unambiguous allele assignment. We have developed assay conditions using novel gel media on the PE Biosystems Prism® 377 DNA Sequencer which result in increased resolution between individual repeat alleles, and dramatically improved run-to-run reproducibility of fragment mobilities and individual repeats at commonly examined loci, such as D1S2667 and D1S2836. These improvements in resolution and reproducibility dramatically enhance the accuracy of automated allele calling suing software packages [e.g. GenoTyper®]. We have also developed protocols for carrying out STR analysis on the 377 using 12 cm long gels, which dramatically reduces the time necessary for analysis, relative to the standard 36 cm gels.

Program Nr: 1218 from the 1999 ASHG Annual Meeting

A complete bacterial clone contig and a partial transcript map of the hereditary paraganglioma critical region on chromosome band 11q23. *B.E. Baysal[1], P. Taschner[2], J.E. Farr[1], C.W. Richard III[3], P. Devilee[2], B. Devlin[1]*. 1) Dept Psychiatry, Univ Pittsburgh Medical Ctr, Pittsburgh, PA; 2) Dept Human Genetics Leiden University Medical Ctr, The Netherlands; 3) Wyeth-Ayerst Research, Radnor, PA.

Hereditary non-chromaffin paragangliomas (PGL, glomus tumors, MIM#168000) are mostly benign, slow-growing tumors of the head and neck region. Pedigrees with this rare genetic disorder display features consistent with genomic imprinting: children of affected fathers develop tumors in an autosomal dominant fashion with age-dependent penetrance liabilities, whereas children of affected mothers remain tumor-free in the majority of cases. Most PGL families show linkage to the *PGL1* locus on chromosome band 11q23. *PGL1* has been confined to an approximate 1.5 Mb critical interval between D11S1986 and D11S1347. We have created a high-resolution physical map of the critical region. First, we have tested regional markers on a set of small-insert and mega-YACs, and obtained an ordered set of densely distributed STSs across the critical region. Then, we used a subset of these STSs to screen an approximately 10-fold BAC library. We obtained 20 overlapping BACs spanning the critical region. We have checked the inserts and *Not*I restriction sites in each BAC by pulsed field gel electrophoresis. We mapped three *Not*I sites inside the *PGL1* critical region. We have isolated the insert ends of a set of minimally overlapping BACs, and created more than 30 new STSs. Three BAC ends contain expressed sequences, and one BAC end contains a processed pseudo-gene. We used the BAC end-derived STSs to confirm the overlap patterns between the clones. The order and overlap of a set of the clones are further confirmed by fiber-FISH analysis (see Taschner et al). We have also tested 50 regionally mapping ESTs/genes against our refined physical map. A total of 18 ESTs/genes mapped within the *PGL1* critical region. Positional analysis of the *Not*I sites and ESTs/genes within the critical region reveals a highly skewed distribution with a distal (telomeric) clustering. The genomic resources created in this study will facilitate the identification of *PGL1*.

Program Nr: 1219 from the 1999 ASHG Annual Meeting

Towards positional cloning of the Bardet-Biedl locus 1 on 11q13. *P.L Beales[1], N. Katsanis[1], D.W. Stockton[1], P.T. Mai[1], L. Baird[2], M. Leppert[2], S. Asakawa[3], N. Shimizu[3], R.A. Lewis[1], J.R. Lupski[1].* 1) Mol. and Human Genetics, Baylor College of Medicine, Houston, TX; 2) University of Utah, Salt Lake City, Utah 84112; 3) Keio University, Tokyo 160-8582.

Bardet-Biedl syndrome (BBS) is a genetically heterogeneous recessive disease primarily characterized by atypical retinitis pigmentosa, obesity, polydactyly, hypogenitalism and mental retardation. Despite the presence of at least five loci in the human genome on chromosomes 2q, 3p, 11q, 15q and 16q, as many as 50% of the mutations appear to map to the BBS1 locus on 11q13. The recessive mode of inheritance and the genetic heterogeneity of the syndrome, as well as the inability to distinguish between different genetic loci by phenotypic analyses, have hindered efforts to delineate the 11q13 region as a first step towards cloning the mutated gene. To circumvent these difficulties we collected a large number of BBS pedigrees of primarily North American and European origin and performed genetic analysis with microsatellites from all known BBS genomic regions. Heterogeneity analysis established a 40.5% contribution of the families to the 11q13 locus and haplotype construction on 11q-linked pedigrees revealed several informative recombinants, defining the BBS1 critical interval between D11S4205 and D11S913, a genetic distance of 2.9 cM, equivalent to approximately 2.6 Mb. Loss of identity by descent in two consanguineous pedigrees was also observed in the region, potentially refining the region to 1.8 Mb between D11S1883 and D11S4944. Using these markers as starting points, we constructed a BAC/PAC contig across the entire critical interval, which serves as a tool for obtaining genomic sequence and for anchoring some 107 cDNA clusters we have identified in the region. We are currently refining the genetic interval further and sequencing genes in search of mutations in BBS1-liniked pedigrees.

Program Nr: 1220 from the 1999 ASHG Annual Meeting

A Primary Transcript Map for the Van der Woude Syndrome (VWS) Critical Region Derived from 900 kb of Genomic Sequence at 1q32-q41. *B.C. Bjork[1], B.C. Schutte[1], M.I. Malik[1], K. Coppage[1], S.G. Gregory[2], D.J. Scott[2], L. Brentzell[3], Y. Watanabe[1], M.J. Dixon[3], J.C. Murray[1]*. 1) Dept. of Pediatrics, Univ. of Iowa, Iowa City, IA; 2) The Sanger Centre, Hinxton, Cambridge, CB10 1SA, UK; 3) Depts. of Dental Medicine and Surgery, Univ. of Manchester, Manchester M13 9PT, UK.

VWS is an autosomal dominant form of clefting with bilateral lower lip pits. The VWS gene was localized to a 1.6 cM region of 1q32 between D1S491 and D1S205. We constructed a sequence-ready BAC contig across the VWS critical region (VWSCR) using gene-based and anonymous STSs from our previous YAC contig. All STSs and BAC clones were shared with the Sanger Centre (U.K.) which developed a contig of 97 PAC clones over the same region. 11 overlapping clones from both contigs, spanning approximately 1.1 Mb, were selected for high-throughput genomic (HTG) sequence analysis. The complete sequence has been generated for 9 of the clones. This 1.1 Mb region is comprised of two contiguous sequences of 721 kb (contains the 350 kb VWSCR) and 129 kb that are separated by a gap of about 300 kb of which 150 kb is partially sequenced. These sequence contigs were analyzed and revealed novel polymorphisms, including an 8 kb deletion/insertion (Watanabe et al., submitted) and 11 STRPs. To identify genes in these sequences, we first used the computer program RepeatMasker2 to "mask" repetitive sequences, then performed BLAST homology searches against the non-redundant and dbEST databases in Genbank. Various gene prediction programs, utilized singly and in combination, with Genotator, were used to identify putative exons in the genomic sequence. These analyses revealed 6 genes previously mapped to the region, 9 novel genes, 8 putative gene fragments and 3 pseudogenes. As they are determined, exon sequences are screened for the presence of etiologic mutations in a panel of 97 affected individuals by SSCV analysis and direct DNA sequencing. To date, 31 normal sequence variants have been identified from this analysis of 92 exons, but no disease-causing mutations were observed. We continue to determine the expression patterns, transcript sizes and cDNA sequence of gene fragments to prioritize genes for mutation analysis.

Program Nr: 1221 from the 1999 ASHG Annual Meeting

Artificial neural network used for the detection of mutations in DNA sequence raw data traces. *L.S. Bjorkesten, T. Soderman.* Informatics and Software, Amersham Pharmacia Biotech, Uppsala, Sweden.

Mutation detection has important applications in many different areas ranging from early drug target discovery to clinical diagnostics. Even though there are many methods available there are few being as general in applicability and defining as accurately the nature of the change as direct DNA sequencing. Depending on the origin of the mutation, one should in the general case expect any mixture between a mutated DNA component and the wild-type component in the sample. Inherited mutations give rise to a heterozygote 50-50 percent mixture while induced mutations e. g. in tumour tissue may give rise to any mixture depending on the heterogeneous composition.

Base-calling strategies are traditionally designed and optimised for genomics oriented applications. The main focus is then on read-length and on the accurate assignment of pure bases. Less effort has been put into the analysis of heterozygote samples and mixtures in general.

Our ANN based mutation detection algorithm is applied as a second pass on data processed by ordinary base-calling software. Every single base position is reconsidered as potentially hiding a point mutation. A number of descriptive features derived from the raw data traces, from the actual sample as well as from a reference sample, are fed into an artificial neural network, which is trained to produce mutation assignments. The performance of the ANN based mutation detection algorithm will be discussed in terms of sensitivity and specificity and it will be pointed out that pre-processing of the descriptive features is essential in order to keep the required training at a reasonable level. Results will be presented where the false positive rate has been reduced by 97% as compared to results obtained using ordinary basecalling strategies.

Program Nr: 1222 from the 1999 ASHG Annual Meeting

Autosomal dominant hearing loss (DFNA18): Physical mapping and analysis of transcripts in the DFNA18 critical region on Chromosome 3q22. *D. Boensch[1,2], C. Neumann[1], C. Weiller[2], A. Lamprecht-Dinnesen[3], T. Deufel[1].*
1) Institut für Klinische Chemie und Laboratoriumsdiagnostik, Klinikum der Universität Jena, Germany; 2) Klinik für Neurologie, Klinikum der Universität Jena, Germany; 3) Poliklinik für Phoniatrie und Pädaudiologie, Universität Münster, Germany.

Investigating a large German pedigree with non-syndromic hearing impairment of early onset and autosomal dominant mode of inheritance, the phenotype was mapped to a 11-cM interval on chromosome 3q22 defined by the markers D3S1589, proximally, and D3S1569, distally, with a maximum two-point lod score of 3.8 for the marker D3S1292; recombination events in a non-affected sib would narrow this to the interval flanked by D3S1664 and D3S3546. DFNA18 is part of a cluster of deafness loci, along with DFNB15 and USH3 from which it has been excluded, and it is located immediately adjacent to, and in fact overlaps by a few markers with, the recently described DM2 locus where hearing loss has been described as one feature of the DM2 phenotype to which, similar as in DM1, the DFNA18 gene might contribute. The entire DFNA18 critical region is comprised in an array of three overlapping YAC clones. In order to identify candidate genes for the hearing disorder we are currently constructing a combined BAC and PAC contig by screening pooled BAC (751 CEPH, Denis Le Paslier) and PAC (704 RCPI1, Pieter de Jong) gene banks obtained through the RZPD (DHGP, Berlin) with known STS markers from the region; additional STS markers have been created by end sequencing of BAC clones. We will present the updated physical map of the region which, at present, consists of several clusters of partial contigs covering the entire region. A number of ESTs have been mapped into the interval, one of them representing a gene for a calcium ATPase, and another two (A005Y32 and WI-16414) obtained from cochlear transcripts of unknown function, and are currently analysed for mutations in affected members of the pedigree.

Program Nr: 1223 from the 1999 ASHG Annual Meeting

Genetic refinement and physical mapping of the *CMT4B* gene on chromosome 11q22. *A. Bolino[1], E.R. Levy[1], M. Muglia[2], F.L. Conforti[2], A. Gambardella[2], E. Le Guern[3], K. Christodoulou[4], A. Quattrone[2], M. Devoto[5], A.P. Monaco[1].* 1) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom; 2) Istituto di Medicina Sperimentale e Biotecnologie, CNR, Cosenza, Italy; 3) INSERM U.289, Hôpital de La Salpêtrière, Paris, France; 4) The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus; 5) Rockefeller University, New York, NY.

Autosomal recessive Charcot-Marie-Tooth (CMT) diseases are a heterogeneous group of disorders in which three different pathological forms have been recognised: one with basal laminar onion bulbs or CMT4A, one with classic onion bulbs or CMT4C, and a third characterised by focally folded myelin sheaths or CMT4B. We recently mapped the *CMT4B* gene on chromosome 11q22 using homozygosity mapping and haplotype sharing on a large inbred Italian pedigree.

In the attempt to identify the *CMT4B* gene, we constructed a YAC contig across the 4 cM critical region between markers D11S1332 and D11S917. Using 19 physically ordered microsatellite markers, we performed a linkage analysis on the original CMT4B family as well as on two additional CMT4B pedigrees. In this way, we confirmed linkage of the *CMT4B* locus to the 11q22 region and, on the other hand, additional recombination events occurring in two affected individuals from the Italian inbred pedigree allowed us to narrow down to approximately 2 Mb the critical region for CMT4B, between markers D11S1757 and GATA3B05.

Then, in order to establish a transcriptional map of the enlarged CMT4B candidate region, we tested on the same YAC contig 70 ESTs retreived from public databases. 54 out of 70 EST clones have been found to be positive by PCR on the YAC clones. In particular, 31 out of 70 EST clones map to the candidate 2 Mb interval and their complete sequence has been determined. Finally, after an extensive computer analysis, we demonstrated that 17 different transcription units as well as 2 already identified genes, which might constitute potential candidates for the *CMT4B* gene, map in the interval delimited by markers D11S1757 and GATA3B05.

Program Nr: 1224 from the 1999 ASHG Annual Meeting

The University of Iowa Gene Discovery Program. *M.F. Bonaldo[1], T.E. Scheetz[1], G. Beck[1], B. Berger[1], C.L. Birkett[3], R. Brown[1], K. Crouch[1], M. Donahue[1], G. Doonan[1], J. Gardiner[1], L. Guo[1], B. Johnson[1], R. Kinkaid[1], K. Liu[3], V. Miljokovic[1], C. Roberts[3], N. Wu[1], T. Casavant[3], V. Sheffield[1], M. Bento Soares[1,2].* 1) Pediatrics, The University of Iowa, Iowa City, IA; 2) Physiology and Biophysics, The University of Iowa, IA; 3) Electrical and Computer Engineering, The University of Iowa, IA.

It is widely recognized that the generation of ESTs from 3' ends of cDNAs constitutes an efficient gene discovery strategy. The main problem with the EST approach is the redundant generation of ESTs derived from the most common RNAs. Accordingly, the use of normalized libraries in which the frequency of all clones is within an order of magnitude range has been shown to expedite gene discovery in large-scale EST programs. However, the process of normalization only contributes to minimize redundancies within (not between) libraries. To address this problem, we developed a new strategy named Serial Subtraction of Normalized Libraries (SSNL). SSNL is an iterative approach whereby all arrayed cDNA clones from a library (typically a complex mixture of individually tagged normalized libraries) are pooled and used as driver in a subtractive hybridization with the library from which they originated. Since the representation of the driver population is significantly reduced in the resulting subtracted library, redundant generation of ESTs is greatly minimized. Most importantly, this process enhances the proportional representation of rare mRNAs likely to be missed in more random approaches. SSNL has been successfully applied in two large-scale gene discovery programs that we are conducting at The University of Iowa: the Rat Gene Discovery and Mapping Program and the Brain Molecular Anatomy Project. To date we have identified 27,000 unique rat 3'ESTs and 9,244 unique mouse 3'ESTs, in a total of only 45,000 rat ESTs and 17,088 mouse ESTs. It is noteworthy that 5,616 of our mouse brain ESTs are not yet represented among the 20,970 members of the existing mouse UniGene set (UniGene build #53). We are beginning to apply the same strategy in an effort to contribute to the gene discovery goals of the NCI Cancer Genome Anatomy Project.

Program Nr: 1225 from the 1999 ASHG Annual Meeting

Using DHPLC to identify disease related polymorphisms in the human Interleukin 4 gene. *M.R. Bonner[1], M. Kabesch[2], F.D. Martinez[2], M.F. Hammer[1]*. 1) ARL Biotechnology, University of Arizona, Tucson, AZ; 2) Respiratory Sciences Center, University of Arizona, Tucson, AZ.

Denaturing High Performance Liquid Chromatography (DHPLC) is a rapid and accurate method for identifying sequence variation in PCR products by detecting mismatched heteroduplexes formed during reannealing of wild type and mutant DNA. PCR product up to 1000bp in length can be screened for single nucleotide polymorphisms (SNPs) at high accuracy when digested with restriction enzymes to create fragments smaller than 600bp. This method was used to search for SNPs in the promoter region of the human Interleukin 4 gene (IL-4), which is involved in the development of allergic diseases. A PCR product covering 925 bp of the IL-4 promoter region and including one previously identified SNP was amplified and digested with RsaI, yielding fragments of 387bp and 538bp, which were then analyzed with DHPLC. Samples showing a change in DHPLC pattern were sequenced to identify the molecular basis of the change. A blinded analysis of a panel of 60 chromosomes correctly identified the mutant and wild type chromosomes for the known SNP. DHPLC also identified a novel SNP in 8 chromosomes in the panel. Sequencing of randomly selected samples showing no change in DHPLC pattern failed to detect sequence variation. From these results we conclude that DHPLC is a sensitive, accurate and fast method for the detection of polymorphisms in candidate genes for human diseases.

Program Nr: 1226 from the 1999 ASHG Annual Meeting

Structure analysis of the PC-1 gene: association of a common amonoacid variant with insulin resistance. *M. Bozzali[1,3], L. Frittitta[2], V. Tassi[1], T. Ercolino[1], A. Argiolas[1], A. Ratti[3], R. Baratta[2], R. Vigneri[2], V. Trischitta[1], B. Dallapiccola[1], A. Pizzuti[1,3].* 1) Endocrinology, Casa Sollievo della Sofferenza, S. Giovanni Rotondo, Italy; 2) Internal Medicine, University of Catania; 3) Neurology, University of Milano.

Insulin resistance (IR) characterizes type 2 diabetes (T2D) and up to 25%; healthy individuals who are at risk to develop for T2D and cardiovascular diseases. PC-1 glycoprotein, inhibits insulin receptor. PC-1 is over-expressed in muscle, fat and fibroblasts of insulin resistant individuals and its over-expression is associated with impaired insulin receptor signaling. Human PC-1 gene is located on chromosome 6q2.2-2.3 near the region for transient neonatal diabetes (TNDM). We determined the PC-1 gene structure. Cosmid subclones from chromosome 6 YACs containing PC-1 sequences allowed to unravel the exon/intron PC-1 gene organization. PC-1 gene is about 50 Kb long with 25 exons. To more finely locate the PC1 gene, PCR analysis of a YAC contig from chromosome 6q2.2-2.3 positioned the PC-1 gene between markers D6S457 and WI-3398, making it an unlike candidate for TNDM. A highly polymorphic CA repeat follows exon 2. All the 25 exons were screened for the presence of polimorphisms. Exon 4 showed a frequent A/C transversion at codon 121 causing a Gln for a Lys change in a Cys-rich domain (K121Q). Q allele frequency in 121 healthy individuals was 17.6%;. The Q carries (39 KQ and 2 QQ) showed IR as indicated by higher glucose and insulin levels during OGTT ($p<0.001$) and by euglycemic glucose clamp ($p<0.01$). Insulin stimulation of receptor tyrosine kinase activity was reduced ($p<0.01$) in cultured skin fibroblasts from 5 KQ in respect to 5 KK healthy subjects. No difference was observed in skeletal muscle PC-1 expression between the Q carriers and KK subjects. PC-1 K121Q is associated to decreased insulin sensitivity in healthy individuals. Therefore, Q allele carriers may be at higher risk to develop diabetes. Our data suggest that PC-1 is an important candidate for the genetic regulation of whole body insulin sensitivity.

Program Nr: 1227 from the 1999 ASHG Annual Meeting

MICROMAX ™:A Unique, Highly Sensitive cDNA Microarray System for Differential Gene Expression Analysis. *B.A. Brown, J. Killian.* NEN Life Science Products, Boston, MA.

cDNA microarray technology is a powerful tool in modern functional genomics research, enabling the study of whole functional pathways or even complete genomes, as opposed to individual genes. NEN Life Science Products, Inc. has developed the first complete, fully validated microarray product system for differential gene expression analysis, through our collaboration with AlphaGene Inc.. The MICROMAX Human cDNA Microarray System I is the first in a line of microarray products designed to provide high throughput and high sensitivity expression profiling for all research lab. The system includes two identical glass microarray slides, each pre-spotted with 2400 known human genes, all key reagents, and a detailed protocol. In this first microarray, 10+ tissue sources are represented, over 40% of which are full-length genes. The 2,400 genes are characterized functionally based on Prosite "motif" search, and are readily identifiable through a gene data base resident on NEN's website, linked to the NIH GenBank.Also included in the system are reagents for cDNA synthesis, hybridization, and NEN's patented, proprietary TSA (Tyramide Signal Amplification) reagents with cyanine 3 and cyanine 5 for signal generation, amplification and detection. TSA reduces the amount of starting sample required by as much as 50-fold, and because of its exceptionally high sensitivity, permits the detection of mRNA expression at levels far below those detectable by other methods. MICROMAX represents a breakthrough in enabling application of DNA microarray technology to a broad research community, particularly in academic and government research institutions. Along with this turn-key product system, NEN also offers slide scanning and data processing services to researchers who do not have access to a laser scanner.

Program Nr: 1228 from the 1999 ASHG Annual Meeting

Improved methods for using gene arrays. *D. Brown, S. Acosta.* Research and Development, Ambion, Inc., Austin, TX., USA.

Although gene arrays are powerful tools for rapidly characterizing thousands of mRNAs within a single sample, cost prohibits their use in many applications. Maximizing the sensitivity and lifespan of an array reduces its effective cost by increasing the information that it can provide. We have found that when using standard hybridization buffers, only 1-5% of any given labeled mRNA binds to its complementary probe on a gene array. Modification of the hybridization buffer allows essentially 100% hybridization without increasing background or cross-hybridization, providing as much as 100-fold greater sensitivity. The lifespans of gene arrays are dramatically reduced by the harsh conditions used to remove labeled nucleic acids prior to reprobing. The boiling SDS washes that are typically used tend to remove the gene sequences covalently bound to the solid support, generally limiting gene arrays to three probings. To reduce the stringency of stripping washes, we have developed probes made with modified nucleotides that are stable under conditions used for hybridization and washing but that can be cleaved with the introduction of a specific reactive agent. Cleavable cDNA probes are incorporated into standard hybridization, wash, and detection protocols, then the hybridized cDNAs are degraded and removed with a mild wash. Forgoing harsh stripping protocols increases gene array lifespans by as much as three-fold. Combining the enhanced hybridization buffer and degradable probes allows 3 to 10-fold more genes to be characterized per sample and as many as 10 mRNA populations to be analyzed per gene array.

Program Nr: 1229 from the 1999 ASHG Annual Meeting

Human Chromosomal Maps of Ancient Sequence Genes. *G.A. Bruns[1,2], R.E. Eisenman[1]*. 1) Genetics Div, Children's Hosp, Boston, MA; 2) Dept Pediatrics, Harvard Medical School, Boston, MA.

By cross-species database searches, we have identified ~100 human genes that have significant homology in the *C.elegans* genome but do not recognize known proteins or protein families. As additional *C.elegans* ORFs remain to be analyzed, ~50 more human genes of this type can be expected. These genes define a small subset of human loci that encode previously unknown ancestral sequences, a number of which can be expected to be "class marking" functional or structural protein domains. We carried out human-specific BlastP searches of GenBank sequences using as bait *C.elegans* ORFs from the WormPep16 database that had no protein identifier. Any high scoring retrieve that recognized a homolog of known function or a member of a function family was excluded. The E values of the 100 new homologs ranged from $5e^{-05}$ to $4e^{-140}$ with the majority having scores of at least e^{-15}. Most of these genes have UniGene cluster numbers with at least 75% of the clusters mapped on GB3/GB4 RH panels. Ideograms of the map positions of these newly recognized ancestral genes are being generated for each of the human chromosomes. The maps are anchored, where possible, to the cytogenetic location of reference loci. These loci, when coupled to available expression data from the EST database and functional analysis in *C.elegans*, become a set of candidate genes for human inherited disorders.

Program Nr: 1230 from the 1999 ASHG Annual Meeting

Transcript map of the Usher syndrome 1C gene locus on chromosome 11p14-15.1. *G.M Caldwell[1], C.D Day[1], P.R Cooper[1], R.L Eddy[1], F. Hejtmancik[3], G.A Evans[4], R.J.H Smith[2], C.C Morton[5], M.J Higgins[1], T.B Shows[1].* 1) Cancer Genetics, Roswell Park Cancer Institute, Buffalo, NY; 2) Department of Otolaryngology, University of Iowa, Iowa City, IA; 3) National Eye Institute, National Institutes of Health, Bethesda, MD; 4) The McDermott Center for Genome Research, University of Texas Southwestern Medical Center, Dallas, TX; 5) Department of Pathology, Brigham and Women's Hospital, Boston, MA.

Usher syndrome 1C (USH1C) is a disorder characterized by congenital hearing loss, absent vestibular function and retinal degeneration. Genetic mapping has assigned the USH1C locus to a region of approximately 400 kb on chromosome 11p14-15.1 between markers D11S902 and D11S1890. To facilitate gene identification, a physical map of this region has been generated using P1 artificial chromosome (PAC) clones. A combination of direct sequence analysis, direct cDNA selection and exon trapping experiments have been used to generate a transcript map of the USH1C region. Fine mapping has localised 22 expressed sequence tags (ESTs), 4 known genes (*NEFA, BIR, SUR1* and *PI3KC2A*) and 5 exons to the region of interest. By virtue of their position, genes mapping to this region represent candidate USH1C loci. The *NEFA* (DNA binding/EF hand/acidic amino-acid-rich) gene, the *PI3K2CA* (phosphatidylinositol 3-kinase, class 2, alpha subunit) gene and the *BIR* (inward rectifier K channel) gene were examined in USH1C patient material using direct sequencing, SSCP and REF but no mutations were found. Several additional transcripts are currently being examined using the same approach. Analysis of the expression pattern and transcript size of novel genes localized within the USH1C region is currently ongoing.

Program Nr: 1231 from the 1999 ASHG Annual Meeting

Variation of individual recombination rates in normal and infertile men. *V.E.H. Carlton[1], D. Nudell[2], M. Castillo[2], N.B. Freimer[1], R. Reijo[2].* 1) Neurogenetics Laboratory, Department of Psychiatry, University of California San Francisco; 2) Department of Obstetrics, Gynecology, and Reproductive Biology, University of California San Francisco, San Francisco, CA, 94122.

Studies in inbred lab strains, such as mouse and fly, have shown that recombination rates differ between members of the same species. Additionally, a significant difference in the recombination rate over a portion of the HLA region has been observed between two normal human males. However, it is not clear how much recombination rates typically vary in normal human males. Inter-individual differences in recombination fraction may have an effect on interpreting genetic maps and on efforts to localize disease genes, in particular efforts using linkage disequilibrium analysis.

Numerous model organisms with defects in genes involved in recombination are infertile. This is unsurprising as recombination is essential for chromosome exchange during meiosis. Additionally, several studies have indicated an increase of non-disjunction (an expected result of recombination defects) in infertile men. Hence we decided to investigate if recombination is abnormal in infertile men.

By genotyping markers in individual sperm from a man, it is possible to calculate individual recombination rates over an interval. We are genotyping sperm from fertile men and from infertile men whose infertility is apparently due to oligospermia (sperm count < 5e6 sperm / ml.) Initially we are genotyping these men with D19S418 and D19S210. These markers span a male recombinational hotspot; a physical distance of less than 1.5 Mb corresponds to a genetic distance of 15 cM in the male and 0 cM in the female. Eventually, other regions will be genotyped. By typing these markers in normal and infertile men we expect to measure how much recombination rates vary in this region in normal human males and to determine if infertile men show evidence for abnormal recombination.

Program Nr: 1232 from the 1999 ASHG Annual Meeting

A physical and transcription map of 1q24.3-q31.1 for the identification of hereditary disease genes including HPC1. *J. Carpten[1], C. Robbins[1], R. Sood[1], T. Bonner[4], I. Makalowska[1], D. Stephan[1], J. Smith[1], N. Scott[1], M. Faruque[1], H. Pinkett[1], C. Graham[1], T. Connors[2], S. Morgenbesser[2], K. Su[2], K. Klingler[2], G. Landes[2], S. Gregory[5], H. Williams[5], W. Isaacs[3], J. Trent[1]*. 1) NHGRI, NIH, Bethesda, MD; 2) Genzyme Genetics Corporation, Framingham, MA; 3) Johns Hopkins Hospital Brady Urological Clinic, Baltimore, MD; 4) NIMH, NIH, Bethesda, MD; 5) Sanger Genome Sequencing Center, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK.

Several hereditary disease loci have been genetically mapped to the chromosome 1q24.3-q31.1 interval including the HPC1 locus. In addition to HPC1, camptodactyly-arthropathy-coxa vara-paricarditis syndrome (CACP), and hyperparathyroid/jaw tumor syndrome (HRPT2) have both been genetically mapped to the 1q24.3-q31.1 region. A 20Mb YAC contig of the 1q24.3-q31.1 has been confirmed by STS content mapping and spans the interval flanked by D1S212 and AFMB330XE9. Over 300 BAC and PAC clones have been identified by PCR screening of commercially available library DNA pools with public and novel markers placed on the YAC clontig. We have generated a complete 6Mb sequence ready BAC/PAC contig of the 1q25.2 interval by STS content mapping and chromosome walking. Approximatley, 130 new STS's have been generated from this contig and shown to map to the 1q25.2 interval, as well as 18 novel simple sequence repeat polymorphisms which are being used for genetic refinement of multiple disease loci. BAC/PAC contig integrity has been confirmed by restriction fingerprinting and clones from this contig are being used as templates for human chromosome 1 genome sequencing. A transcription mapping effort has resulted in the precise localization of 18 known genes and 31 EST's by database searching, exon trapping, direct cDNA hybridization, as well as sample sequencing of BACs from the 1q25.2 region. An additional 11 known genes and ESTs have been placed within the larger 1q24.3-q31.1 interval. These transcription units represent candidate genes for multiple hereditary diseases including the HPC1 gene.

Program Nr: 1233 from the 1999 ASHG Annual Meeting

Superiority of Denaturing High Performance Liquid Chromatography (DHPLC) over single-strand conformation polymorphism (SSCP) and conformation-sensitive gel electrophoresis (CSGE) for mutation detection in TSC2. *Y.S. Choy[1, 2], S.L. Dabora[1], F. Hall[1], V. Ramesh[3], Y. Niida[3], D. Franz[4], J. Kaprzyk-Obara[5], M.P. Reeve[1], D.J. Kwiatkowski[1]*. 1) Genetics Laboratory, Heme Div, Brigham & Women's Hosp, Boston, MA; 2) Division of Genetics, Children's Hospital, Boston, MA; 3) Molecular Neurogenetics Unit, Mass Genl Hosp, Boston, MA; 4) Divison of Pediatric Neurology, Children's Hospital Medical Center, Cincinnati, Ohio; 5) Department of Child Neurology, Children's Memorial Hospital, Warsaw, Poland.

Tuberous sclerosis (TSC) is an autosomal dominant tumor suppressor gene syndrome with a wide mutational spectrum in two causative genes, TSC1 and TSC2. We evaluated DHPLC as a scanning method for mutation detection in TSC2, and compared it to CSGE and SSCP. The first 20 exons of TSC2 were amplified from 84 TSC patients and screened initially by CSGE and then by DHPLC. PCR products with gel shifts or DHPLC changes were evaluated by automated sequencing. Optimization of DHPLC analysis of each exon was carried out by design of primers with minimum variation in the melting temperature of the amplicon, and titration of both elution gradient and temperature. CSGE analysis identified 40 shifts (21 unique) in the 84 patients and 20 exons. All of these same variants were detected by DHPLC, and an additional 27 changes (14 unique) were identified. Overall 15 of 28 (54%) unique single base substitutions were detected by CSGE; all were detected by DHPLC. 25 definite or probable mutations were found in these 84 patients (30%) in exons 1-20 of TSC2. In a subsequent blinded analysis of 15 samples with 19 distinct sequence variants originally detected by SSCP in another center, all variants were detected by DHPLC except one where the variation occurred within the primer. Ten other (7 unique) sequence variants were detected in these samples which had not been detected by SSCP. Overall, 12 of 19 (63%) unique single base substitutions were detected by SSCP; all were detected by DHPLC. We conclude that DHPLC is superior to both CSGE and SSCP for detection of DNA sequence variation in TSC2.

Program Nr: 1234 from the 1999 ASHG Annual Meeting

From Sequence Analysis To Diseases Identification In the Distal Human Xq28. *A. Ciccodicola, T. Esposito, M.R. Matarazzo, F. Gianfrancesco, M. Cocchia, M. Cuccurese, T. Bardaro, M. Manfellotto, M.G. Miano, C. Migliaccio, M. Vacca, A. Franze', S. Cocchia, G. Mercadante, A. Terracciano, A. Torino, V. Ventruto, M. D'Esposito, M. D'Urso.*
International Institute of Genetics and Biophysics, CNR, Naples, Italy.

The distal Xq28 region is an interesting region for its gene content, its association with genetic diseases, for the presence of the second pseudoautosomal region (XqPAR) with its unusual biology and for the Xq telomere. We have sequenced the last 500 kb of this region. By using six different algorithms to predict putative exons (GRAIL II, GENEFINDER, GENEID, GENESCAN, GENIE and FGENEH programs) and BLAST programs to search for EST matches, we show the presence of 5 genes (BBOX2; HSPRY3; SYBL1; IL9R and XAP139), 3 putative genes predicted by software analysis and one pseudogene (AMD2) in the last 500 kb of the Xq28 region. Of the three new genes (BBOX2, HSPRY3, and XAP139) one of them, BBOX2, is located in the terminal X-specific 100 kb portion, and it is homolog to a C.elegans gene. The other two genes are located in the XqPAR and, at least for HSPRY3 we have demonstrated allele specific silencing. Molecular analysis are in progress to associate these genes with disease of genetic origin mapped in this reghion, such as IP2 and Rett syndrome.

Program Nr: 1235 from the 1999 ASHG Annual Meeting

An integrated genetic and physical map of the region containing a QTL for HPFH on chromosome 6q23. *J.P. Close[1], L. Game[1], P. Stephens[1,4], J. Mitchell[1], S. Best[1], J. Rochette[2], A. Mungall[3], G. Bethel[3], S. Humphray[3], J. Riley[4], C.G. See[4], P. Sanseau[4], C. Louis-dit-Sully[1], R. Cox[1], L. Kearney[1], S.L. Thein[1]*. 1) Molecular Hematology, Institute of Molecular Medicin, Oxford, UK; 2) Genetique Medicale, Faculte de Medecine, Amiens, France; 3) The Sanger Centre, Hinxton, Cambridgeshire, UK; 4) Glaxo-Wellcome Research and Development, Stevenage, UK.

Fetal hemoglobin (Hb F) and F cell levels in adults show considerable variation and are influenced by several genetic variants, the major determinants appear to be unlinked to the b globin gene cluster. Genetic studies have recently assigned a trans-acting locus (QTL) controlling Hb F and F cell production on chromosome 6q23 in an Asian Indian kindred that includes individuals with heterocellular hereditary persistence of fetal hemoglobin (HPFH) associated with b thalassemia. The initial candidate interval of ~4 Mb was defined by the markers D6S408 and D6S292 in the 6q22.3-q23.2 region. Haplotypes of the 6q markers were constructed; key recombination events now place the QTL within a 1-2 cM interval spanning ~1.5 Mb between the markers D6S270 and D6S1626/D6S292. The refined candidate interval is spanned by 3 YACs: 917-C-6, 750-C-11 and 905-B-7, and covered by overlapping bacterial clones that consist of 231 BACs and 106 PACs ordered by STS content mapping and confirmed by restriction fragment fingerprinting. The minimum tiling path consists of 22 BAC and 1 PAC clones. 150 STSs map to this interval of 1.5 Mb giving an average STS density of ~1/10 kb. About half of the STSs are novel, all generated from end sequence of the BACs and PACs. The contig includes three microsatellite polymorphic markers, ten SNPs and seventeen unique loci identified as expressed sequence tags (ESTs) of which five relate to characterised genes. The resources should greatly facilitate the construction of a transcript map and sequence analysis of this region leading to the identification of the QTL controlling Hb F/F cell production.

Program Nr: 1236 from the 1999 ASHG Annual Meeting

Fine genetic and physical mapping of the second gene associated with Marfan Syndrome (MFS2). *G. Collod-Beroud[1], G. Jondeau[2], M. Coulon[1], G. Delorme[2], O. Dubourg[1], J-P. Bourdarias[2], C. Junien[1,3], C. Boileau[1,3].* 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.

Marfan Syndrome (MFS) was the founding member of the "heritable disorders of connective tissue". The cardinal features of this autosomal dominant syndrome are prominently observed in 3 systems: skeletal, ocular and cardiovascular. It is now well established that defects in the fibrillin gene located on chromosome 15 (FBN1) cause not only classic MFS, but also a large range of milder, overlapping phenotypes. We have been investigating a large family of more than 200 subjects with a connective-tissue disorder. The autosomal dominant phenotype segregating in this family associates anomalies in the skeleton (tall stature, arm span greater than height, arachnodactyly, scoliosis, and pectus), the heart and the aorta (mitral valve prolapse, aortic dilation, aortic dissection or rupture), skin and integument. None of the ocular features observed in classic MFS was documented in the family until ectopia lentis was identified in a young girl from the last generation. By excluding the FBN1 locus in this large 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 previously localized MFS2 to a region of less than 7 cM between D3S1293 and D3S2335 which map at 3p24.2-p25. The analysis was enlarged to 20 new family members (7 in the last generation). Four new dinucleotide DNA markers (D3S3473, D3S3700, D3S3598, and D3S3659 from Gnthon map) and 4 tetranucleotide DNA markers (D3S3038, D3S2466, D3S3047 and D3S1759 from CHLC) were tested in the family. These data refined the genetic localization of MFS2 to area of less than 5 Mb. This region, contained within a single YAC, has an estimated physical distance of less than 1,4 Mb. Combination of data from the different integrated maps shows less than 50 potential cDNA transcripts. Of these, there are no known genes that could make plausible candidates.

Program Nr: 1237 from the 1999 ASHG Annual Meeting

The gene of branchio-oculo-facial syndrome does not colocalize to the EYA1-3 genes or the branchio-oto-renal syndromeé. *L. Correa-Cerro[1,2], M. Dietmar[3], W. Just[1], W. Vogel[1], I. Kennerknecht[1]*. 1) Dept Medical Genetics, Univ, Ulm, Germany; 2) Doctorado en Genetica Humana, Univ, Guadalajara, Mexico; 3) Klinikum Chemnitz, Saeuglingsklinik, Chemnitz, Germany.

The branchio-oculo-facial syndrome (BOFS) is an autosomal dominant disease characterized by branchial cleft sinus or linear skin lesion behind the ear, colobomata of the iris and/or retina, lacrimal duct obstruction and auricular and lip-pits, hypertrophy of the lateral pillars of the philtrum, a broad, asymmetric nose with a broad tip. It has been postulated that BOFS could be an allelic disease to the branchio-oto-renal (BOR) and branchio-oto (BO) syndromes. The BOR gene has been localized on chromosome band 8q13.3. It was named EYA1, the human homologue of the drosophila gene eyes absent. BOR and BO are allelic defects of the EYA1 gene. In order to test the hypothesis that BOFS is also an allelic disease with BOR and BO, we studied the co-segregation of four microsatellite markers on chromosome 8q with the disease in the largest family with BOFS described in the literature. Genomic DNA was prepared from peripheral blood lymphocytes using standard procedures and the different segments were amplified by PCR. The analysis was performed on 7 members of the family including 5 affected individuals using the Allelic links software from Pharmacia Biotech. The results were exported to Cyrillic program for pedigree drawing. The segregation of the haplotypes was determined using SimWalk, the resulting haplotypes reentered into Cyrillic and evaluated for co-segregation with BOFS. The markers used here did not co-segregate with the disease. The study was extended to markers defining two other genes of the EYA family, EYA2 on chromosome 20q13.1 and EYA3 on chromosome 1p36.1-36.2. In both cases co-segregation could also be excluded. Thus, we conclude that the BOF syndrome is not allelic to BO and BOR and that the gene responsible for BOFS remains unknown. We will continue with other markers in order to find a possible candidate region for BOFS.

Program Nr: 1238 from the 1999 ASHG Annual Meeting

Applying rapid DNA microarray optimization capability to SNP screening and genotyping. *M.T. Cronin, F. Frueh, M. Pho, D. Dutta, T.M. Brennan.* Protogene Laboratories, Inc, Palo Alto, CA.

Developing large-scale discovery and testing capability for genetic variability is critical as emerging pharmacogenetic data provide increasingly detailed genotype-phenotype associations and improve phenotype prediction from composite genotypes. Such data have great potential to diminish inter-individual variability in response to xenobiotics. Genetic profiling is expected to increasingly influence and individualize pharmacotherapy optimization, thereby reducing potential for adverse drug reactions and risk from environmental exposures. However, providing individual pharmacogenetic assessment means analyzing many genes for complex mutation and polymorphism patterns. This task poses a significant challenge to current genotyping strategies.

DNA microarrays provide an efficient tool for broadly accessing polymorphism information. We have developed FlexChip™DNA microarrays to provide a highly versatile format for nucleic acid analysis, including polymorphism screening and genotyping. By using piezoelectric jet reagent delivery to array features defined by surface tension, two types of DNA arrays are made quickly and economically. Off-the-shelf, DMT-phosphoramidite chemistry has been successfully adapted for *in situ* synthesis of oligonucleotide probes up to 50 bases long on these surface tension substrates. In addition, pre-synthesized PCR products, cloned cDNAs or purified oligonucleotides are covalently immobilized using surface tension to direct localization on array surfaces.

Using NAT2 gene polymorphisms as a biomarker for drug-response and metabolism, we have successfully demonstrated the high synthesis quality and design flexibility of our array system. We have used on-the-fly array design capability to optimize microarrays to scan NAT2 sequences for evidence of polymorphisms and for accurate SNP genotyping. This is a crucial step since the rapid advancement of human genome sequencing provides new gene sequences and informative new polymorphic markers on nearly a daily basis. Results from NAT2 and other genetic analysis models will be presented.

Program Nr: 1239 from the 1999 ASHG Annual Meeting

Exclusion of the b'**-COP gene and identification of a novel candidate gene for the blepharophimosis syndrome (BPES).** *E. De Baere[1], E. Roman[1], Y. Fukushima[2], K. Verhoeven[3], G. Van Camp[3], A. De Paepe[1], L. Messiaen[1]*. 1) Department of Medical Genetics, University Hospital Ghent, Ghent, Belgium; 2) Division of Medical Genetics, Saitama Children's Medical Center, Saitama, Japan; 3) Department of Medical Genetics, University of Antwerp, Antwerp, Belgium.

BPES is a rare genetic disorder presenting with blepharophimosis, ptosis and epicanthus inversus. BPES type I is associated with female infertility. A disease locus has been mapped to 3q23. Recently, we have narrowed the BPES critical interval to 45 kb by the identification of a cosmid spanning the 3q23 breakpoint in a BPES patient carrying a balanced t(3;4)(q23;p15.2) translocation.

The b'-COP gene has been mapped to 3q23, distal but in close proximity to this translocation breakpoint. Hence, this gene can be considered to be a positional candidate for BPES. The total coding region of the b'-COP gene was analysed in 10 unrelated BPES patients by direct sequencing of the cDNA and Southern blot analysis using 3 cDNA probes spanning the entire gene. As no mutations nor rearrangements were found, we provide evidence that this gene can be excluded as a candidate gene for the BPES syndrome.

Shotgun cloning and sequencing of the cosmid spanning the 3q23 breakpoint revealed the presence of a known EST, derived from a testis cDNA library. 5' and 3' RACE PCR on the EST resulted in the cloning of a transcript of 2.5 kb. By comparison of the cDNA sequence obtained by RACE PCR with the genomic sequence of the assembled cosmid contigs, 3 exons/2 introns have been determined. No significant homologies with known genes have been identified until now. Southern blot hybridisation with the RACE products on the t(3;4) translocation patient revealed a rearrangement, suggesting that this gene is disrupted by the translocation. By its position, this novel gene is an important candidate for BPES. Cloning of the full-length cDNA, Northern studies and mutation analysis in BPES patients are currently underway in order to assess its possible role in BPES.

This study is supported by the Fund of Scientific Research-Flanders (FWO).

Program Nr: 1240 from the 1999 ASHG Annual Meeting

A new genome wide screening of 281 affected subjects in 84 families with adult-onset Primary Open Angle Glaucoma (POAG). *T. Desai[1], D. Van Hoewyk[1], I. Stoilov[1], A. Child[2], G. Brice[2], M. Sarfarazi[1].* 1) Molecular Ophthalmic Genetics; Surgical Research Center, Department of Surgery, University of Connecticut Health Center, Farmington, CT; 2) Department of Cardiological Sciences, St. George's Hospital, London, UK.

The purpose of this study was to identify new chromosomal locations in families with adult onset Primary Open Angle Glaucoma (POAG) that do not map to the six previously known loci of GLC1A->GLC1F. A random genome wide screening of 281 subjects with adult onset POAG in 84 families was performed. Approximately 83% of these families have between 3-7 affected members. Our strategy was to screen the genome with 169 markers at 20 cM intervals, followed by another 217 intervening markers and at a final resolution of 5-10 cM. Using an ABI-877 Integrated thermal cycler and/or GenAmp 9700 systems, multiplex PCR reactions were carried out. The amplified products of 8-12 markers were loaded onto 96 well gels on an ABI-377 DNA sequencer. The results were evaluated with GENESCAN and GENOTYPER softwares. The genotypes obtained during this screening were evaluated using both parametric (2-point and multipoint LOD scores; haplotype transmission data) and non-parametric (sib pairs) methods of linkage analysis. As a result of this screening, we obtained LOD score values of over 2.00 (ranging between 2.04 to 2.73) for 5 new locations on chromosomes 1, 7, 16, 18 and 19. LOD scores of over 1.00 (ranging between 1.08 to 1.91) have also been observed for other locations on chromosomes 3, 11, 12, 14, 17 and 21. However, linkage to all of these new loci still needs to be confirmed. This is currently being done by saturation mapping, additional genotyping of normal subjects and by further evaluation of haplotype transmission data of both normal and affected individuals within these families. Positive identification and confirmation of new loci for POAG will be a major contribution to the overall understanding of the genetics, complexity and heterogeneity of primary open angle glaucoma. Supported by NIH (EY-09947), IGA (G249) and InSite Vision, Inc.

Program Nr: 1241 from the 1999 ASHG Annual Meeting

Physical and EST mapping on chromosome 9: Candidate Genes for the geniospasm (GSM1) locus. *P.H. Dixon[1], V.J. Stinton[1], S.J. Humphray[2], S. Hammond[2], P.J. Howard[2], I. Dunham[2], N. Wood[1], M.B. Davis[1].* 1) Neurogenetics Unit, Institute of Neurology, London, England; 2) The Sanger Centre, Hinxton Hall, Cambridge, England.

Hereditary geniospasm is a movement disorder characterised by episodes of involuntary tremor of the chin and lower lip. The condition presents early in childhood, and may be precipitated by stress, concentration and emotion. A recent genome-wide scan in a four-generation family, with geniospasm inherited as an autosomal dominant trait, revealed linkage to a 2.1cM region on the long arm of chromosome 9, band 9q13-q21, between the markers D9S1806 and D9S175. The GSM1 critical region appears to span a region of 3 megabases, based upon a YAC map of the region. Here we describe progress to physically characterise this region with a detailed small-insert contig (PAC/BAC clones) ready for long-range sequencing as part of the chromosome 9 sequencing project, and the localisation of brain-specific ESTs to this region, which are being investigated as candidate genes for hereditary geniospasm. A database screen has revealed the presence of 12 ESTs isolated from brain cDNA libraries that map to this region. Confirmation of the localisation of these ESTs is underway, using the YAC contig, together with PACs and BACs from the critical region. BLAST analysis of each of these potential candidates has revealed no significant homologies to previously characterised sequences. Thus, our analysis describes progress towards a sequence-ready physical map of this region and has identified candidate loci for the GSM1 gene on chromosome 9.

Program Nr: 1242 from the 1999 ASHG Annual Meeting

Draft and Finished Sequencing of Human Chromsome 16. *N.A. Doggett, M.O. Mundt, D.C. Bruce, A.C. Munk, D.L. Robinson, M.D. Jones, J.M. Buckingham, L.A. Chasteen, E.H. Saunders, L.A. Goodwin, A.L. Williams, J.L. Longmire, P.S. White, L.L. Deaven.* DOE Joint Genome Institute, Center for Human Genome Studies, Los Alamos National Laboratory, Los Alamos, NM 87545.

We are pursuing a plasmid-based approach toward the large scale sequencing of human chromosome 16. Our strategy for sequencing involves nebulization to randomly break DNA, size selection of 3 kb and larger fragments, double adapter cloning into plasmid, and sequencing of both ends up to 4.5X random sequencing coverage. Assembly of sequence contigs is assisted with JAVA tools which exploit the inherent relationship of the paired-end sequences. Closure and finishing is achieved by a combination of primer walking, shatter libraries, longer reads, and alternate chemistry reactions. We have recently introduced the use of the Mermade 96 channel oligonucleotide synthesizer which enables us to reduce the level of shotgun in favor of targeted primer walks. We have also begun double-end plasmid draft sequencing to 4X coverage in Phred Q20 bases, producing ordered and oriented contigs. Drafting accelerates the sequencing process, and we have completed over 2 Mb in this format during the past few months. To date we have submitted more than 5.2 Mb of finished sequence, including a 3.0 Mb contig from 16p13.3 spanning four disease genes-- TSC2, PKD1, MEFV and CREBP. G+C content is high across the region, with a maximum of 57% found in a finished clone. Gene density approaches one gene/10 kb in some stretches. Alu content has also been high, with up to 30 Alus in a finished cosmid. Sequence comparison analysis is semi-automated with use of the SCAN program (developed at LANL). This program launches a suite of sequence similarity searches and gene prediction algorithms and summarizes the results into a single integrated annotation report. The quality of finished sequence is ensured by stringent criteria including; > 95% of bases with Phrap scores > 40 and > 95% of bases covered in both strands (or 2 chemistries), assembly validation by restriction fragment analysis with 3 enzymes, and post submission quality assessment.
Supported by the US DOE, OBER.

Program Nr: 1243 from the 1999 ASHG Annual Meeting

Chromosomal localization of novel cDNAs using real-time fluorescent PCR (TAQMAN) on radiation hybrid panels. *P. Dowd, M. Roy, A.D. Goddard.* Molecular Biology, Genentech, Inc., So San Francisco, CA.

The construction of a human gene map is a key step in the identification of disease-causing genes and is important in understanding the organization of the genome. The use of radiation hybrid (RH) panels to localize loci on framework maps is an efficient method for accomplishing this task. A PCR based approach to testing gene-specific STSs on the G3 RH panel (http://www-shgc.stanford.edu /Mapping/index.html) and the Genebridge4 RH panel (http:/www-genome.wi.mit.edu/ cgi-bin/ contig/rhmapper.pl) has been used to localize approximately 500 novel genes. Initial mapping accomplished by analyzing PCR products from the hybrid panels on agarose gels has been replaced by real-time fluorescent. The application of the TaqMan real-time PCR system (ABI PRISM 7700, Perkin-Elmer-Applied Biosystems) to screening RH panels has increased our throughput, sensitivity and accuracy. The coupling of gene-specific PCR primers with a nested dual-labeled fluorogenic hybridization probe increases the specificity of the reaction by removing signal from non-specific PCR products which do not hybridize to the internal, labeled probe. The TaqMan reactions are characterized by the cycle at which the amplification product is first detected above a threshold (CT) rather than the amount of PCR product accumulated after a fixed number of cycles providing us with rapid and sensitive detection of product. We have used chromosomal location data to aid in the identification of candidate genes and in the analysis of disease-related genomic alterations.

Program Nr: 1244 from the 1999 ASHG Annual Meeting

Signing on to Expression Profiles. *M.M. Dressman[1], E. Holland[2], M.H. Polymeropoulos[1], C. Lavedan[1]*. 1) Pharmacogenetics, Novartis Pharmaceuticals, Gaithersburg, MD; 2) MD Anderson Cancer Center, Houston Texas.

Unique expression patterns of a large number of genes can now define cell specific signatures under a given context. Cell specific signatures will be invaluable to understanding differentiation, cell type specificity and the cellular biology of environmental responses. Expression signatures could greatly enhance drug development by increasing efficacy and decreasing toxicity of therapeutic compounds. We have used (Affymetrix) oligonucleotide microarrays to survey and compare the expression profiles of 7000 human genes from cells of various origins, including lymphocytes, umbilical vein endothelial cells (HUVEC), a breast adenocarcinoma cell line (MCF7) and a glioblastoma cell line (U373MG). Environmental context such as time in culture, state of differentiation, media composition, CO2 content and culture confluency may alter cell signatures. Chemical compound manipulation of cell lines provides one mechanism to dissect function at the gene expression level. For example, extended treatment of endothelial cells with vascular endothelial growth factor (VEGF) results in greater than two fold changes in expression level of ~100 genes, while extended treatment of glioma cell lines with platelet derived growth factor (PDGF) results in greater that two fold changes in 39 genes. Analyzing expression data from multiple cell lines can also be used to identify similarities of expression profiles for all cell lines. We have observed that an average cell type expresses 2335 out of 7000 genes assayed (~ 33 %). Sixty-four percent of the 2335 genes are expressed in endothelial, U373MG, and MCF7 cells, with a total of 630 expressed at similar levels in all three cell types despite variation in growth media. Additional cell types are being analyzed to refine the universal and cell-specific signatures.

Program Nr: 1245 from the 1999 ASHG Annual Meeting

Further gene identification in the *dn* critical region. *S.S. Drury[1], D. Scott[2], D. Arnaud[1], J. Bishop[2], R. Poche[1], M. Batzer[1], M. Lovett[3], V. Sheffield[2], R. Smith[2], P. Deininger[2], B. Keats[2].* 1) Genetics & Biometry, LSU Sch MEd, New Orleans, LA; 2) Department of Pediatrics, Univ. of Iowa, Iowa City, IA; 3) University of Texas Southwestern Meidcal Center, Dallas, TX.

The deafness locus (*dn*) has been mapped by linkage analysis to mouse chromosome 19. The orthologous region on human chromosome 9 contains the DFNB7/11 locus for autosomal recessive nonsyndromic hearing loss and it is likely that the *dn* gene and the gene causing DFNB7/11 are homologues. The *dn* mouse is a naturally occurring mutation in the curly-tail (*ct*) stock and homozygous *dn/dn* mice have profound sensorineural hearing loss with degeneration of the organ of Corti and the stria vascularis. No other phenotypic abnormalities exist in the mutant mouse. Recombination data and flourescent in situ hybridization (FISH) studies strongly support the existence of an inversion on the *dn* chromosome relative to the *ct* chromosome that is hypothesized to have caused the deafness phenotype. One side of the inversion is contained on BAC 124J3 and we are screening a subclone contig of this BAC for rearrangements at the genomic level indicative of the breakpoint. Several other ESTs, identified with exon trapping, mapping to this region, are also being examined. We have mapped three genes to the *dn* locus and excluded them as candidates genes using Northern analysis, rtPCR, and direct sequencing. These genes included TMEM2, a member of the Arrestin family, and ZNF216. Additionally, we have examined the known genes that map to this region, including lipocortin, and have found no mutations in the *dn* mouse. To date, no mutations have been found in the DFNB7/11 families in any of these genes.

Program Nr: 1246 from the 1999 ASHG Annual Meeting

Fine structure mapping of the hereditary inclusion body myopathy locus. *I. Eisenberg[1], T. Levi[2], A. Sanilevich[1], M. Shemesh[1], H. Hochner[1], M. Sagi[5], J.G. Seidman[2], M. Sadeh[3], Z. Argov[4], S. Mitrani-Rosenbaum[1].* 1) Unit for Development of Molecular Biology and Genetic Engineering, Hadassah Hospital, Hebrew Univ.-Hadassah Medical School, Jerusalem, Israel; 2) Dept. of Genetics, Harvard Medical School, Boston; 3) Dept. of Neurology, Wolfson Hospital, Holon; 4) Dept. of Neurology; 5) Dept. of Genetics, Hadassah Hospital , Jerusalem, Israel.

We have previously mapped the gene responsible for the recessive form of Hereditary Inclusion Body Myopathy (HIBM) in Persian Jews to a 10cM interval on chromosome 9p1-q1, and showed that the disease is linked to the same locus in other ethnic groups such as Afghani and Iraqui Jews, and a non Jewish family from India. Also, Nonaka et al., have shown that an HIBM type of the disease described in Japanese patients links to the same locus. In order to narrow the gene locus and identify putative candidate genes for HIBM, we have extended our linkage studies to 40 families using two-point linkage and linkage disequilibrium analyses, and used yeast and bacterial artificial chromosome (YAC and BAC) libraries to physically map the HIBM gene.

Linkage analysis and recombination events allowed us to define the HIBM interval to a 1cM region. Furthermore, we have identified microsatellite markers which are in strong linkage disequilibrium with the disease. b tropomyosin, a skeletal muscle protein which maps within this region, was excluded as the disease causing gene because an intragenic polymorphism did not exhibit linkage disequilibrium in HIBM probands. Subsequently we have identified a YAC insert, about 1 megabase long, which contains both markers flanking the HIBM interval. Exon trapping of this insert allowed us to identify 2 novel genes. We have established the exon/intron structure of these genes which are being screened for putative mutations causing HIBM. Furthermore, data from the most recent integrated genomic map (GeneMap98) revealed that more than 10 new ESTs physically map to this region.

We conclude that the HIBM gene resides in a 1 megabase interval on chromosome 9 and speculate that a novel muscle protein encoded there is mutated in HIBM.

Program Nr: 1247 from the 1999 ASHG Annual Meeting

High resolution physical and transcript mapping of the critical interval for the ARSACS gene identifies two candidate genes. *J.C. Engert[1], C. Doré[1], J. Mercier[2], P. Berubé[1], P. Lepage[1], B. Ge[1], K. Morgan[1], T.J. Hudson[1], A. Richter[2]*. 1) Centre for Genome Research, Research Institute, Montreal General Hospital; 2) Service de Génétique Médicale, Centre de Recherche Mére-Enfant,Hôpital Sainte-Justine, Montréal, Québec,Canada.

Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS, OMIM 270550) is an early onset neurodegenerative disease associated with prominent myelinated retinal nerve fibers. The high prevalence of ARSACS in the Charlevoix-Saguenay region of Quebec (carrier frequency of 1/22) and rarity elsewhere is indicative of a founder effect. In a previous study we localized the disease gene to chromosome region 13q11 by identifying excess sharing of a marker allele in patients. Haplotype reconstruction revealed a major ancestral haplotype and an unrelated minor one. To create a detailed physical map of the ARSACS region, we screened CEPH mega-YACs with 41 chromosome 13 STSs mapping to 13q11-q12. The YAC contig, composed of 27 clones, extends from D13S175 to D13S221. A high resolution BAC and PAC map was constructed that includes the ARSACS critical region flanked by D13S1275 and D13S292. These YAC and BAC/PAC maps allowed the accurate placement of 29 genes and ESTs previously mapped to the proximal region of chromosome 13q. We confirmed the position of two candidate genes (SGCG and KIAA0730) within the ARSACS critical interval, and mapped the 27 other genes and ESTs to nearby intervals. Six BAC/PAC clones form a contig between D13S232 and D13S787, the 450 Kb critical interval defined by fine structure linkage disequilibrium mapping. Sample sequencing of M13 subclones obtained from these BACs and PACs identified six new EST clusters in addition to the two known transcripts. Sequencing of additional M13 subclones, and extension of these known ESTs will allow us to characterize the candidate genes for ARSACS.

Program Nr: 1248 from the 1999 ASHG Annual Meeting

Analysis of 7 Mb from human chromosome 21: Gene identification relevant to Down syndrome and features of genome organization. *K. Gardiner, D. Slavov, R. Lucas, C. Reimer, K. Clancy, A. Rynditch.* Eleanor Roosevelt Inst, Denver, CO.

To identify novel genes of relevance to Down syndrome and to investigate potential regulatory sequences, we are analyzing the chromosome 21 sequence generated by centers in Japan and Germany. To date, annotation of >7 Mb, dispersed throughout the long arm, has consisted of exon prediction with four programs, protein and EST database searches, 2-sequence Blast searches and CpG island identification, followed by RT-PCR for verification and refinement of predicted novel gene models. In this analysis, 40 well characterized or related genes were found. Success of exon prediction for these genes suggests that this analysis alone should reveal >90% of genes. However, within the same 7.5 Mb, novel genes (those lacking any protein homology) are being predicted with the following characteristics: a) exon + EST genes: 4 genes with excellent patterns of predicted exons and one or more matches in dbEST; b) exon - EST genes: 20 genes with good or marginal patterns of predicted exons and no matches in dbEST; c) EST - exon genes: 7 genes without patterns of reliable exon prediction but with matches in dbEST; d) CpG island genes: 11 genes consisting of strong CpG islands that are apparently unique sequences and found in >50 kb regions lacking any consistent exon predictions. For a subset of novel genes, RT-PCR and RACE experiments helped to verify and refine the models, and were used to define expression in early development and in adult brain regions potentially relevant to Down syndrome. These also revealed examples of complex alternative processing, especially in brain, that may have important implications for regulation of protein function. To begin identification of regulatory sequences, complete gene structures were determined for the 40 known genes. This identified a number of very large introns, a number of very short intergenic distances, and at least one potentially bidirectional promoter. Relating observed gene size and gene density to the isochore model of the mammalian genome also permits a refined estimate of the total number of genes on chromosome 21.

Program Nr: 1249 from the 1999 ASHG Annual Meeting

Concerted evolution within and between rDNA clusters on human acrocentric chromosomes. *I.L. Gonzalez[1], J.E. Sylvester[2]*. 1) Research Department, A.I. Dupont Institute, Wilmington, DE; 2) Nemours Children's Clinic, Jacksonville, FL.

It is known that the human rRNA genes (rDNA), which are clustered on the 5 acrocentric chromosomes, contain sequence variation not only in the intergenic spacer, but also in the coding regions. However, due to the concerted evolution that operates on tandemly-repeated genes the rDNA are much more similar than would be expected of interspersed repeats. We examined the question of how similar are rDNA copies within single clusters and compared this to homologous and non-homologous intercluster similarity, by cloning and sequencing rDNA fragments derived from specific acrocentric chromosomes isolated in somatic cell hybrids. The fragments include 28S coding sequences, intergenic spacer, regulatory regions upstream of the promoter, and segments of adjacent non-rDNA. Our results indicate: [1] Preferential homogenization within clusters, where differences among clones are usually of the same magnitude as the pcr error rate. [2] rDNA from homologous chromosomes are not more similar than rDNA from non-homologues. [3] While regulatory and coding regions show very low divergence among all chromosomes examined, the intergenic spacer of some of the chromosomes is 10 times more divergent. [4] Sequences from the terminal rDNA are slightly diverged from those of internally-located rDNA repeats. [5] All acrocentrics examined have the same distal junction to non-rDNA. [6] Sequences distal to rDNA are surprisingly homogeneous. [7] rDNA pseudogene fragments located in the distal region do not evolve in concert with parent sequences in rDNA. We conclude that intracluster homogenization of rDNA is very efficient and much more prevalent than homologous and non-homologous exchanges.

Program Nr: 1250 from the 1999 ASHG Annual Meeting

GENE EXPRESSION PROFILING IN HUMAN TISSUES OF HYPOTHALAMUS-PITUITARY-ADRENAL AXIS BY EXPRESSED SEQUENCE TAGS (ESTs) AND FULL-LENGTH cDNA CLONING. *Z. Han[1], R. Hu[2], H. Song[2], Y. Peng[1,2], Q. Huang[1,2], S. Ren[1], Y. Gu[1,2], C. Huang[1], Y. Li[1], C. Chang[1], G. Fu[1], Q. Zhang[2], B. Gu[2], T. Wu[1], Y. Yu[1], S. Xu[1], S. Fu[1], M. Chen[2], J. Chen[2], Z. Chen[1,2]*. 1) Chinese National Human Genome Center at Shanghai, Shanghai,China; 2) Shanghai Institute of Endocrinology, Rui-Jin Hospital,Shanghai Second Medical University, Shanghai,China.

Hypothalamus-pituitary-adrenal (HPA) axis is an essential part of neuroendocrine system. To obtain a first overview of transcriptional genomics in this axis, genes expressed in human hypothalamus (HT), pituitary (NP), and adrenal gland (AD) were catalogued by generating a large amount of expressed sequence tags (ESTs), followed by bioinformatics analysis. Totally, 25973 sequences of good quality were obtained out of 31130 clones (83.4%) from cDNA libraries of the three tissues (HT, NP and AD). After eliminating 5329 ESTs corresponding to repetitive sequences and mitochondrial DNA, 20644 ESTs could be assembled into 9176 clusters when overlapping and/or redundant ESTs were integrated. 2778 clusters (30.3%) corresponded to known genes, 4165(44.8%) to dbESTs, while 2233 (24.3%) to novel ESTs. The gene expression profiles reflected very well the functional characteristics of the three levels in HPA axis, with myelin basic protein, growth hormone and steroidogenic acute regulatory protein genes as most highly expressed in HT, NP and AD, respectively. Subsequently, 150 full length cDNAs of novel genes were cloned and sequenced, which may be involved in critical life processes such as gene expression, processing and transport of secretory proteins, signal transduction, metabolism, development, and differentiation. In addition, some new findings were made with regard to the physiology of HPA axis, including the existence of steroidogenic enzyme gene transcription in hypothalamus, the high expression of human osteoinductive factor in pituitary possibly as a new pituitary hormone, and a local CRH-ACTH-cortisol system in AD. Moreover, mRNA isoforms as a result of alternative splicing were found in several genes, including the growth hormone gene. All these data may contribute to the understanding of the neuroendocrine regulation of human life.

Program Nr: 1251 from the 1999 ASHG Annual Meeting

Collection of informative SNPs by PLACE-SSCP analysis of pooled DNA. *K. Hayashi, T. Sasaki, M. Inazuka, A. Suzuki, Y. Kukita, T. Tahira.* Div. Genome Analysis, Inst. Genet. Inf., Kyushu Univ, Fukuoka, Fukuoka, Japan.

A high-resolution genome map densely marked with informative single nucleotide polymorphisms (SNPs) is expected to be a powerful tool for genome-wide association studies of polygenic traits. However, an extensive work is needed to collect many such markers, and suitable new strategy/technique is preferred to alleviate the task. We previously developed a polymorphism/ mutation detection method, PLACE-SSCP, in which PCR products are fluorescently post-labeled and separated by automated capillary electrophoresis in SSCP conditions (Inazuka et al., Genome Res. 7, 1094-1103,1997). This method is scalable, involving minimal human intervention. We show here, that PLACE-SSCP analysis of pooled DNA is efficient in finding informative SNPs, because in the analysis, SNP-allele frequencies in the pool are accurately estimated from heights of separated peaks. We started from 48 STS on chromosome 6, which are reported to contain 61 candidate SNPs (Wang et al., Science 280, 1077-1082, 1998). Two ethnic groups (53 Japanese and 78 Caucasian) were examined by the method, and all STSs in selected individuals were sequenced to verify the SNPs. We found that; (1) 17 of the 61 SNPs (28 %) were likely to be monomorphic, (2) heterozygosity of 11 of quantified 34 SNPs (32 %) was less than 20% in both populations, and (3) 12 of the quantifiable 34 SNPs (35 %) had noticeably different allele distribution between the two populations. We also found 11 new SNPs in the examined 51 STSs. In the present method, allele frequencies of 34 of the 44 confirmed SNPs (77 %) were quantifiable. Software for resolving and quantifying overlapped SSCP peaks is being developed, which should enable quantification of 90 % of the SNPs.

Program Nr: 1252 from the 1999 ASHG Annual Meeting

Construction of a PAC contig in the putative 7q32 imprinted region flanking *MEST/PEG1*. *S. Hayashida, Y. Asada, K. Yamasaki, T. Kishino, N. Niikawa.* Human Genetics, Nagasaki Univ. Sch. of Med., Nagasaki, JAPAN.

MEST/PEG1, a gene expressed paternally in mesodermal derivatives in early embryonic stages, is the first imprinted gene mapped to chromosome 7q32. Loss of MEST function is thought to contribute to intrauterine and postnatal growth retardation associated with maternal uniparental disomy for chromosome 7 (mUPD7). Since imprinted genes are clustered in general at a chromosomal region, we speculate that a similar imprinted-gene cluster may exist at 7q32 region and functions of some such genes may contribute to the phenotype of mUPD7. As an initial step toward the isolation of imprinted genes at 7q32, we adopted an integrated approach involving construction of a PAC contig and EST mapping in the vicinity of *MEST*. PAC clones covering *MEST* were first isolated by PCR-based screening of a PAC library and end-sequencing of the framework PACs allowed further isolation of adjacent PACs to construct a ~1Mb PAC contig spanning *MEST*. Regions with only one-fold coverage and several small gaps in the contig were filled with BAC clones. Ordering of PACs in the contig was confirmed by interphase FISH and a YAC contig spanning the region of *D7S530*, *MEST* and *D7S649*. Using these genomic sources, ESTs from "Gene Map 99" were localized by PCR. This allowed us precise mapping of 12 ESTs including 3 known genes (*UBE2H,CPA1* and *CPA2*) in the contig. In the course of construction of the contig, BLAST searches with end-sequences of PACs identified three previously unmapped ESTs. The PAC contig constructed and ESTs mapped precisely will help us to analyze imprinting of genes including *MEST* at 7q32.

Program Nr: 1253 from the 1999 ASHG Annual Meeting

Construction of a physical and transcriptional map of human chromosome 17p13.3 to identify a new tumor suppressor gene implicated in breast cancer. *C. Hoff[1], P. Seranski[1], T. Detzel[1], B. Korn[1], J. Ramser[2], R. Reinhardt[2], U. Hamann[1], A. Poustka[1]*. 1) Molecular Genome Analysis, German Cancer Research Center, Heidelberg, Germany; 2) Maxplanck institut for molecular genetic, Berlin, Germany.

Chromosome 17 aberrations like gene amplifications and loss of heterozygosity (LOH) are the most common genetic abnormalities in sporadic forms of human breast cancer. Although LOH mapping studies on 17p in breast tumors showed that the common LOH region contains the p53 gene in 17p13.1, mutations in p53 have been found in only 17-40 % of the investigated cases. Existence of LOH in breast cancer tumors distal to TP53 suggests that there is a new tumor suppressor gene in 17p13.3. LOH confined to 17p13.3 has also been found in medulloblastoma, astrocytoma, ovarian cancer, bladder cancer and osteosarcoma. We are constructing an integrated physical map in 17p13.3 with overlapping sets of yeast artificial chromosome (YACs), P1 derived artificial chromosome (PACs) and cosmids covering approximately a 1,5 Mb interval of sequence ready map. Focusing on the specific region of interest observed through LOH analysis using three polymorphic markers on 60 breast tumors, we have continued our work by transcript mapping on the precisely mapped clones. So far 30 ESTs have been identified and fine mapped, most of them show no homology to known sequences. In parallel the complete genomic sequence of the region is in progress. This allows the detection of additional potential coding sequence using exon prediction algorithms like GENESCAN and GRAIL. Structure / functional based approaches and expression profiling will allow the rapid identification of new tumor suppressor candidate genes. Profiling in normal breast and tumor samples is based on Northern blot hybridization and RT-PCR on the one hand and on complex hybridisations of EST specific PCR products on mini arrayed nylon filters on the other hand.

Program Nr: 1254 from the 1999 ASHG Annual Meeting

Microdeletions in the Y chromosome in idiopathic infertile men. *M.J.V. Hoffer[1,2], J.W.A. de Vries[2], B. Redeker[1], S. Repping[2], L.G. Brown[3], D.C. Page[3], J.M.N. Hoovers[1], F. van der Veen[2], N.J. Leschot[1]*. 1) Dept. of Clinical Genetics, Academic Medical Center, Amsterdam, The Netherlands; 2) Center for Reproductive Medicine, Dept. of Gynaecology and Obstetrics, Academic Medical Center, Amsterdam, The Netherlands; 3) Howard Hughes Medical Institute, Whitehead Institute and Dept. of Biology, Massachusetts Institute of Technology, Camebridge, MA, USA.

Molecular genetic analysis in infertile men have shown the existence of three different regions of Azoospermia Factors (AZF-a, -b and -c) in intervals 5 and 6 of the human Y chromosome. Deletions in one of these regions can cause spermatogenic defects ranging from azoospermia to mild oligozoospermia. Recently, a fourth region, AZF-d, has been described (Mol. Reprod. Dev. 1999;53:27) which is located between the AZF-b and AZF-c region. 20% of the microdeletions was found in the AZF-d region and seem to be associated with a less severe phenotype, i.e. mild oligozoospermia or even normal sperm count associated with abnormal sperm morphology. Therefore, we have screened 200 infertile men (undergoing ICSI) using sequence tagged sites (STSs) as well as gene specific markers. The markers used included 12 STS markers located in all the AZF regions and 6 markers for the X/Y homologous genes on the long arm. By means of this strategy, we found one deletion within AZF-b and 4 within AZF-c. We found no deletions of the AZF-a and d region. In conclusion, the AZF-c region and not the AZF-d region seems to be the most interesting one for studying the etiology of male infertility.

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Program Nr: 1255 from the 1999 ASHG Annual Meeting

Computational methods for evaluating clone sampling efficiency in normalized cDNA libraries. *J. Hoh[1], D. Gordon[2], T. Lints[2], T.C. Matise[2], J. Ott[2].* 1) Columbia University, New York, NY; 2) Rockefeller University, New York, NY.

Large-scale gene expression analysis using cDNA microarrays promises to yield new insights into the biological properties at the molecular level. Such microarrays can be constructed from normalized cDNA libraries derived from tissues of particular interest (TOI) (Library I), or from more complex libraries in which a substantial proportion of all genes expressed by the organism are represented (Library II). Library I provides a more efficient means to sample the genes in TOI, whereas Library II is valuable to investigators with broader interests. Here, we develop quantitative methods to assess the relative efficiency of sampling from these two libraries.

To perform this analysis, we address the following 2 problems: 1) how many clones from the tissue-specific normalized library (I) must be sampled in order to acquire a specified proportion of the genes expressed in the TOI? 2) how many clones from the more complex normalized library (II) must be sampled in order to acquire a specified proportion of the genes expressed in the TOI and, in so doing, what proportion of the genes expressed in other tissues (non-TOI) would be acquired? For each of problems 1 and 2, we have developed analytical expressions for the probabilities of acquiring all genes expressed in TOI for a given sample size (the solution is reminiscent of, but not identical to, the well-known coupon collecting problem in combinatorics). Computer simulation will be applied to investigate more general questions. We will assume both perfect normalization (each gene in the library has the same frequency) and more realistic normalizations where the frequency of each gene falls within, for example, a 10-fold or 100-fold range.

Program Nr: 1256 from the 1999 ASHG Annual Meeting

A format for the collection of accurate and useful data on sequence variation. *O. Horaitis, R.G.H. Cotton, & 32 Members of the HUGO Mutation Database Initiative.* Mutation Research Centre, 7th Floor Daly Wing, 41 Victoria Pde, Fitzroy VIC 3065, Australia, Tel: 61-3-9288-2980, Fax: 61-3-9288-2989, cotton@ariel.ucs.unimelb.edu.au.

Disease causing genes are being described with increasing frequency as the Human Genome Project progresses. Similarly disease causing mutations are described at increasing rates such that some single gene disorders are now known to be caused by hundreds of mutations e.g. 870 for the CFTR gene causing cystic fibrosis. If we assume that the number of genes in the genome are 100,000 and that there are 100 changes per gene causing inherited disease and cancer, it is likely that there will be 10 million disease causing events eventually described. However, polymorphisms may be around one third this level. This information must be documented accurately and completely so that the many user groups have confidence in it. **The HUGO Mutation Database Initiative (MDI) (http://ariel.ucs.unimelb.edu.au:80/~cotton/mdi.htm)** has been considering the quality control problem so that it can make a recommendation for collection of data concurring mutational change. Extensive consultation with MDI members has developed an internet based user interface for entry of allelic variations into databases. The most important questions to ensure the mutation described is certain or likely to cause disease are as follows:

- 1. Is the change found in a 2nd PCR sample amplified from the patient's DNA?
- 2. Is the change always present when the phenotype is present?
- 3. Is a conserved residue affected?
- 4. Has expression analysis been performed?
- 5. Have 50 normal chromosomes been tested?

Many other questions relating to detection methods, RNA, etc. are also asked, some relating to the linkability with other databases such as providing a reference sequence and accession number. Reasons for these will be explained. The complete form will be shown and the final format recommended at the next MDI meeting on 19th October 1999.

Program Nr: 1257 from the 1999 ASHG Annual Meeting

Transcript mapping of the Usher syndrome type 3 region on chromosome 3q21-q25. *T.H. Joensuu[1,2], R.H. Hämäläinen[1,2], A-E. Lehesjoki[1,2], A. de la Chapelle[2,3], E-M. Sankila[1,4].* 1) Department of Medical Genetics, Haartman Institute, University of Helsinki, Helsinki, Finland; 2) Department of Molecular Genetics, The Folkhälsan Institute of Genetics, Helsinki, Finland; 3) Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio; 4) Department of Ophthalmology, Helsinki University Hospital, Helsinki, Finland.

Usher syndrome type 3 (USH3) is characterized by autosomal recessive inheritance, progressive bilateral sensory neural hearing loss, retinal degeneration, and variable vestibular dysfunction. We previously assigned an USH3 locus to 3q21-q25 by linkage and refined its localization to a 1-cM region between markers D3S1299 and D3S3625. In order to identify the USH3 gene, we constructed a bacterial artificial chromosome (BAC) contig between the flanking markers D3S1299 and D3S1279. Novel polymorphic markers were generated from the BACs, and used to genotype USH3 families to further narrow the critical region. By analyzing linkage disequilibrium and historical recombinations in 79 Finnish USH3 chromosomes the location of USH3 was refined to a less than 250 kb genomic interval. A number of STSs generated from the isolated BAC ends and novel ESTs have been localized to the map. Four out of seven ESTs, which mapped within the minimal USH3 region, were found to represent two separate genes, namely SIAH2 and KIAA0001. These were studied as potential candidate genes for USH3 and excluded as causative by sequencing the complete coding regions in patients. Further analysis of the remaining ESTs is ongoing. BACs covering the critical region are being sequenced and a complete transcript map is under construction in order to identify the USH3 gene.

Program Nr: 1258 from the 1999 ASHG Annual Meeting

Synteny between human chromosome 18q and mouse chromosome 18 extends throughout the 18q- syndrome dysmyelination critical region. *T.L. Johnson-Pais[1], C.L. Buller[2], R.J. Leach[1,2].* 1) Department of Pediatrics, University of Texas Health Science Center, San Antonio, TX; 2) Department of Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, TX.

Common features of the human chromosome deletion syndrome 18q-, resulting from hemizygosity of a portion of 18q, include dysmyelination of the central nervous system (CNS), mental retardation, and short stature. Because breakpoints for this syndrome vary, a 2Mb critical region for dysmyelination in 18q23 has been defined by genotype/phenotype mapping. This critical region includes the gene for myelin basic protein (MBP); however, mouse models have shown that loss of MBP alone is not sufficient to cause dysmyelination. Thus, we want to create a "deletion" mouse model missing the region homologous to the human dysmyelination critical region. In order to create a model that has a deletion of MBP and surrounding genes, we have to determine whether synteny exists between human and mouse in the dysmyelination critical region. Important evidence that this region of chromosome 18 is conserved between human and mouse is that the MBP gene and the gene for the galanin receptor 1 (GALR1) are tightly-linked on both human chr 18q23 and mouse chr 18. We have mapped the mouse homologs for ESTs (or complete genes) that have been localized to human chr 18q23 in the dysmyelination critical region with the use of the Jackson Laboratory (C57BL/6JEi x SPRET/Ei)F1 x C57BL/6JEi (BSB)) and (C57BL/6JEi x SPRET Ei/F1 x SPRET Ei (BSS)) interspecies backcross mapping panels. We have shown that the mouse homologs of at least 2 additional genes and 4 ESTs from 18q23 map to the telomeric region of mouse chr 18. We are currently localizing the mouse homologs of 6 additional human ESTs that map to 18q23. This extended region of synteny, which includes ESTs that map both inside and outside the dysmyelination critical region, proves that this region of the genome is well-suited for the creation of a mouse model that will lack the same genes deleted in humans with dysmyelination and 18q- syndrome.

Program Nr: 1259 from the 1999 ASHG Annual Meeting

Genomic sequence analysis in mouse t-complex region. *G.J. Kargul[1], P. Waeltz[1], P. Ma[2], E.Y. Chen[2], M.S.H. Ko[1], R. Nagaraja[1]*. 1) Laboratory of Genetics, National Institute on Aging, Baltimore, MD 21224; 2) Celera Genomics, 850 Lincoln Center Drive, Foster City, CA 94404.

A systematic analysis of 10 Mb of the mouse t-complex region on chromosome 17 has been undertaken to define the content of genes involved in early embryonic development. Mapping and sequence analysis are complicated by the presence of a number of large duplications and inversions in this region. We have initiated the effort to construct an unambiguous STS/BAC based physical map by integrating newly mapped markers (11 Wsu and 16 Ertd markers). We are also identifying corresponding BAC clones for other markers already placed in the t-complex region, making additional STSs from inter-B1 products of individual BACs, and integrating additional genetic data available for the region to complete a physical map and resolve duplicated regions. Thus far, three BACs spanning approximately 600 kb have been sequenced and are being analyzed. The region, syntenic with human 16p13, contains 28 STSs integrated with 33 overlapping BAC clones. In one stretch of 175 kb, genomic sequence were found that encode the transcripts for Nubp2 (Nucleotide binding protein 2; corresponding to EST, D17Wsu11e) and D17Wsu15e. The same sequence also contains the known but previously incompletely mapped Als (which binds and stabilizes the IGF/IGFBP3 complex in serum to extend its half-life), as well as the nucleoside diphosphokinase 3 genes and the 3'end of the Rsp29 gene. Five additional potential coding regions with CpG islands would yield an overall average of at least one gene every 18 kb, making this region of the t-complex part of the highest gene density fraction of the genome.

Program Nr: 1260 from the 1999 ASHG Annual Meeting

Identification of retina-specific transcripts through in silico subtraction and construction of a genome-wide retinal gene map. *N. Katsanis[1], P. Culpepper[1], T. Thangarajah[2], C. Weber[2], M. James[2], J.R. Lupski[1], K. Worley[1]*. 1) Mol and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Wellcome Institute, Oxford, UK.

Since the inception of the EST project, the numbers of partial sequences in Genbank have increased exponentially, reaching over 1.5 million human sequences from >100 tissue sources and developmental stages. However, it is difficult to examine large numbers of ESTs and address biologically important questions because of the lack of specialized software and other technically limiting factors. Although advances in microarray technology offer new possibilities for high throughput gene expression studies, the number of sequences that can be spotted and evaluated with this technology remains limited compared to the vast numbers of unique sequences in dbEST. We are interested in identifying new retina-specific genes, map them in the human genome and perform expression analyses. To achieve our goal, we have used some 50,000 retinal ESTs that have been deposited in dbEST by designing a package of programs that process all retinal ESTs and extract sequences only when they are found in retinal cDNA libraries and no other tissue, thus increasing the probability that these sequences represent retina-specific transcripts. Through this *in silico* subtraction we identified some 950 sequences potentially representing novel genes, in addition to some 150 sequences derived from known transcripts such as ABCR, RETGC, RPE65, Rhodopsin and CRX. We have mapped these genes in the human genome with the Genebridge 4 radiation hybrid panel to create a retinal transcript map, onto which we have superimposed all known uncloned retinopathies, thus generating a plethora of candidates for these disorders. We have integrated our findings into a bipartite database, RetBase, consisting of the mapping and sequence information of all unique EST clusters (RetMap) and the expression database (RetExpress), both of which will be shortly available to the scientific community. Our approach serves both as a powerful tool in disease gene identification for eye-related diseases as well as a prototype for transcript maps of other tissues.

Program Nr: 1261 from the 1999 ASHG Annual Meeting

Molecular Cloning, Tissue Specific Expression and Assignment to Chromosome 13q12 of Human Connexin 30, GJB6, a Candidate Gene for Nonsyndromic Hearing Loss. *P.M. Kelley[1], S. Abe[2], J.W. Askew[1], S.D. Smith[3], S-i. Usami[2,4], W.J. Kimberling[1]*. 1) Dept Genetics, Boys Town National Research Hospital, Omaha, NE 68131; 2) Dept. of Otorhinolaryngology, Hirosaki University School of Medicine, Hirosaki 036-8562 JAPAN; 3) Center for Molecular Genetics, Munroe-Meyer Institute, University of Nebraska Medical Center, Omaha, NE 68198; 4) Dept. of Otorhinolaryngology, Shinshu University School of Medicine, Matsumoto 390-8621 JAPAN.

Mutations in connexin 26 are responsible for approximately 20% of genetic hearing loss and 10% of all hearing loss. However, only about 75% of the mutations predicted to be in Cx26 are actually observed. While this may be due to mutations in noncoding regulatory regions, an alternative hypothesis is that some cases may be due to mutations in another gene immediately adjacent to Cx26. Another gap junction gene, connexin 30, is found to lie on the same PAC clone which hybridizes to chromosome 13p12. Human connexin 26 and connexin 30 are expressed in the same cells of the cochlea. Cx26 and Cx30 share 77% identity in amino acid sequence but Cx30 has an additional 37 amino acids at its C terminus. These considerations led us to hypothesize that mutations in Cx30 might also be responsible for hearing loss. Sixty recessive nonsyndromic hearing loss families from both European and Japanese populations were screened for mutations. In addition, 21 dominant hearing loss families were tested. No significant mutation has been found in the dominant or recessive families.

Program Nr: 1262 from the 1999 ASHG Annual Meeting

A complete map of 80 human ribosomal protein genes. *N. Kenmochi[1], T. Uechi[1], S. Higa[1], M. Sakai[1], M. Yoshihama[1], N. Maeda[1], D.C. Page[2], T. Tanaka[1].* 1) Dept. of Biochemistry, School of Medicine, University of the Ryukyus, Okinawa, Japan; 2) Howard Hughes Research Laboratories at Whitehead Institute/MIT, Cambridge, MA.

Although the ribosome is essential for cell growth and development, the effects of ribosomal mutations and their role in human disease have been explored barely. However, it has been recently shown that mutations of an rp gene (*RPS19*) cause congenital anemia and, in *Drosophila*, the quantitative deficiency of any of a number of ribosomal proteins results in *Minute* phenotypes, including reduced body size, diminished viability and fertility, and short, thin bristles. Thus, we are assuming that rp gene defects could cause various kinds of human diseases.

To explore this possibility, we have been systematically mapping the human rp genes and have finally completed the map including all the 80 distinct genes. Because localization of rp genes is complicated by the existence of processed pseudogenes, multiple strategies were devised to identify PCR-detectable STSs at introns. The developed STSs were subjected to radiation hybrid panels (GeneBridge4 and G3) and all has been placed quite precisely on the physical map of the human genome. We localized 56 previously unmapped rp genes, and refined or corrected 24 previously reported map positions. Though functionally related and coordinately expressed, the human rp genes are widely dispersed; both sex chromosomes and 20 autosomes (all but chromosome 7 and 21) carry one or more rp genes. Chromosome 19, known to have a high gene density, contains an unusually large number (13) of rp genes. This map provides a foundation for the study of the possible roles of ribosomal protein deficiencies in chromosomal and Mendelian disorders.

Program Nr: 1263 from the 1999 ASHG Annual Meeting

Revealing of rDNA deleted monomers and short disperced rDNA homologous sequences in human genome. *P.M. Kirilenko, N.S. Kupriyanova, A.P. Ryskov.* Dept. of Genom Organization , Institute of Gene Biology, Moscow, Moscow, Russia.

Recently, analysing rDNA containing clones in cosmid library of human chromosome 13 we have found variants harboring extended deletions flanked by (tc)n microsatellite clusters in intergenic ribosomal spacer (rIGS). Now we try to find similar deletions in a total cellular human rDNA. We have performed a series of PCR amplifications for a number of unrelated individuals with 20-mer primers corresponding to the borders of 25kb deletion in one of the rIGS cloned variants. For all individuals similar PCR patterns have been obtained revealing a number of fragments one of which showed a size analogous to the cloned deleted rDNAvariant and ability to hybridize with identical markers. It can signify an existence of sought for deleting variants in human cellular rDNA. Analysis of the other amplificants after their cloning and sequencing revealed a complex mixture of chimeric rDNA-non rDNA sequences as well as nonribosomal DNA of distinct types originating from different chromosomes. It can mean an existence of short rIGS homologous sequences dispersed throughout human genome.

Program Nr: 1264 from the 1999 ASHG Annual Meeting

Genomic sequencing and identification of 39 genes on human chromosome 21. *J. Kudoh[1], K. Shibuya[1], K. Kawasaki[1], A. Shintani[1], K. Nagamine[1,2], J. Wang[1], S. Asakawa[1], S. Minoshima[1], N. Shimizu[1].* 1) Dept. Mol. Biol., Keio Univ. Sch. Med., Tokyo, Japan; 2) Dept. Cell Technol., Eiken Chemical, Tochigi, Japan.

We have been analyzing genomic sequence of human chromosome 21. Our targets are 4 regions in the 21q (approximately 6.5 Mb in size); 1.2-Mb region containing CBR1, SIM2, and HLCS, 440-kb region containing ETS2, 3-Mb region between MX1 and D21S171, and 2-Mb region between APP and D21S292. We use a shotgun sequencing strategy in combination with primer walking and nested deletion methods (Genome Res., 7:250-261, 1997) to completely determine the sequence of each BAC, cosmid, and PAC clone. We have determined approximately 3.5-Mb of sequence with estimated accuracy of >99.999% and will finish sequencing of the entire 6.5-Mb region by the end of 1999. Genomic sequence was subjected to homology search and analysis for protein coding potential using GRAIL and GENSCAN. Putative exon sequences were used as probes to screen corresponding cDNA clones or as primers to amplify cDNA fragments by PCR. Nucleotide sequence of these cDNA clones and those registered in databases allowed us to identify 13 new genes including CBR3, C21orf5, SIM2, PDE9A, KNP1 (Keio novel protein 1), KNP3/TRPC7, KNP5/C21orf2, AIRE, DNMT3L, NR4, NR7, NR8, and LPAAT3, and 26 known genes including CBR1, KIAA0136, CAF1A, HLCS, ERG, ETS2, MX2, MX1, TMPRSS2, WHITE, TFF3, TFF2, TFF1, NDUFV3, PKNOX1, CBS, U2AF1, CRYAA, KIAA0179, PDXK, CSTB, NNP-1, TMEM1, PWP2, KIAA0653, and PFKL. The sequence information is available through our Web site (http://www.dmb.med.keio.ac.jp) or Web site (http://www-alis.tokyo.jst.go.jp) of Japan Science and Technology Corporation (JST) which should be useful for further finding of new genes.

Program Nr: 1265 from the 1999 ASHG Annual Meeting

A physical map of the atopy locus on human chromosome 13q14. *N.I. Leaves, G.G. Anderson, S. Bhattacharyya, Y. Zhang, R. Cox, W.O.C.M. Cookson.* Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, England, OX3 7BN.

Atopy is common familial syndrome underlying asthma. It is a complex genetic trait that is characterised by raised Immunoglobulin E (IgE) responses to common allergens.We have previously identified a region on chromosome 13q14 that is linked to atopy and to total serum immunoglobulin A. Linkage to the region has been replicated by us and other groups. We have now typed 60 published microsatellite markers within the region and shown linkage to atopy in three panels of subjects comprising a total of 604 sibling pairs. The locus shows a maximum lod score >5, with a 1 lod support interval of <5cM. We have constructed a physical YAC STS content map to cover the entire area. The map comprises of 41 STSs and 17 microsatellite markers. YACs were sized by pulsed field gel electrophoresis and hybridization with total human DNA. All YACs were analysed by FISH for chimerism. The YAC contig spans approximately 6Mb and consists of 10 clones with an average insert size of 1.3Mb and an average depth of 1.5 clones. A PAC/BAC contig was then established to cover the peak of linkage in the central 1.5Mb of the YAC map. This contig was constructed using 10 published STSs and 60 novel STSs generated from clone ends by anchor bubble PCR. All PACs and BACs were confirmed as hybridizing uniquely to 13q14 by FISH. The PAC/BAC contig was integrated into the YAC map using the common STSs and by forming a *Not*I and *Sal*I pulsed field restriction map. The PAC/BAC contig contains 38 clones with an average insert size of 114Kb. The contig has an average depth of 2.8 and spans approximately 1.5Mb. Anchor bubble PCR was then used to identify novel microsatellite markers from the PAC/BAC map. Twenty-five microsatellites were recovered, eight of which were polymorphic and were typed in our panels. Genetic analysis showed all markers were strongly linked to atopy. Markers within a central 600kb also showed positive TDT tests to atopy and the serum IgE level. Gene recovery is now in progress using cDNA selection and the eighteen ESTs confirmed as mapping onto the PAC/BAC contig.

Program Nr: 1266 from the 1999 ASHG Annual Meeting

Regional mapping panels for human chromosomes 1, 2, and 7. *J.C. Leonard, L.H. Toji, P.K. Bender, C.M. Beiswanger, J.C. Beck, R.T. Johnson.* NIGMS Human Genetic Cell Repository, Coriell Cell Repositories, Coriell Institute for Medical Research, Camden, NJ.

The NIGMS Human Genetic Mutant Cell Repository is establishing a collection of well-characterized somatic cell hybrid regional mapping panels for each human chromosome. With the cooperation of its advisers and various chromosome committee members, the CCR has identified regional mapping panels for chromosomes 1, 2, and 7 from the hybrids in the collection submitted by S. Bodrug, D. Callen, D. Cox, B. Emanuel, T. Glover, K. Grzeschik, V. vanHeyningen, D. Ledbetter, G. Scherer, T.B. Shows, J. Trent, D. Warburton, and J. Wasmuth. These panels consist of hybrids that define 8 to 10 intervals for each chromosome.

Hybrids were characterized by the submitters; recharacterization performed at the CCR supported the submitters' descriptions of the hybrids. The human chromosome content of the hybrids was confirmed by G-band analysis and *in situ* hybridization with total human DNA and in some cases, with specific chromosome paints or alpha satellite probes. In addition, DNA samples prepared from the hybrids were characterized by Southern blot hybridization and/or by PCR with probes or primers for each human chromosome in the specific human derivative or deletion chromosome.

The NIGMS Human Genetic Cell Repository now has regional mapping panels for chromosomes 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 13, 15, 16, 17, 18, 21, 22, and X. These panels are available as cell cultures or as DNA.

Program Nr: 1267 from the 1999 ASHG Annual Meeting

Identification of differentially expressed genes in human Th2 cells. *X. Li[1], T. Beaty[2], S. Huang[1].* 1) Clinical Immunology, Johns Hopkins University, Baltimore, MD; 2) Department of Epidemiology, School of Hygiene and public health, Johns Hopkins University, Baltimore, MD21205.

Identification of differentially expressed genes in human Th2 cells Xiaodong Li1, Terri H. Beaty2, and Shau-Ku Huang1 1.Division of Clinical Immunology, Asthma and Allergy Center, 2. Department of Epidemiology, Johns Hopkins University School of Medicine, Baltimore, MD 21224 T heper 2(Th2) cells are associated with allergic diseases, including asthma, and are potential targets for developing novel therapies. The mechanisms governing the development of Th2 cell phenotype are currently unclear, and the exact signaling pathways leading to Th2-dominant immune response remain to be determined. To this end, we have developed an efficient and reliable method to identify differentially expressed genes associated with the activation of Th2 cells. This was accomplished by combining suppressive subtractive hybridization (SSH) and high throughput cDNA array analysis. The use of SSH to equalize high and low copies of transcripts and to remove common sequences between tester and driver cell populations allows us to generate probes significantly enriched for differentially expressed genes. We used allergen-stimulated human Th2 cells as tester, and either resting Th2 cells or stimulated Th1 cells as driver populations. Genes differentially expressed in the tester but not in the driver could be examined directly by the use of cDNA microarray. A database is being generated to contain lists of differentially expressed genes of Th2 cells at different time points after activation by allergen exposure.

Program Nr: 1268 from the 1999 ASHG Annual Meeting

Genomic organization and characterization of a human MSUD locus (E1a gene of BCKAD complex) on chromosome 19q13.2: mapped to a single BAC clone. *L.D. Love-Gregory[1], J.R. Newton[2], R.E. Hillman[1,2,3], C.L. Phillips[1,2,3]*. 1) Genetics Area Program; 2) Department of Biochemistry; 3) Department of Child Health, Division of Medical Genetics, University of Missouri-Columbia, Columbia, MO.

Maple Syrup Urine Disease (MSUD), an autosomal recessive metabolic disorder, results from defects in the multienzyme branched chain a-keto acid dehydrogenase (BCKAD) complex (incidence 1/200,000). In Old Order Mennonite communities, MSUD estimated at 1/176 live births, results from a tyrosine to asparagine substitution (Y438N formerly Y393N) in the BCKAD E1a gene (attributed to a founder effect). We found 19% of non-Mennonite MSUD patients also have the Y438N allele (either homozygote or compound heterozygote).

To determine if the prevalence of the Y438N E1a allele in non-Mennonite MSUD patients is the result of a founder effect, we propose to evaluate microsatellite markers tightly linked to the E1a gene (chromosome 19q13.2). In an effort to refine the genomic map of the E1a locus, to establish informative microsatellites for accurate haplotype analysis, and to characterize genomic organization, we screened a human BAC library (Research Genetics, Huntsville, AL) using E1a exon 9 sequence to isolate BAC clones containing the entire E1a gene and its flanking regions. Presently, we have 1) identified two overlapping BAC clones, 228O8 and 123G5 (approximatetly 130 Kb each), 2) determined by southern blot hybridization to exons 1, 4, 5, 7, and 9 sequences and PCR amplification of exons 4, 7, and 9 sequences that BAC 228O8 contains exons 5-9 of the E1a gene, and BAC 123G5 contains the entire E1a gene (exons 1-9, 55 Kb), and 3) determined by southern blot analysis the presence of at least one CA-repeat within BAC 123G5, which is close to the E1a locus. Presence of the entire BCKAD E1a gene and CA repeat(s) within BAC 123G5 will allow us 1) to continue to refine the genomic map of this locus within chromosome 19q13.2, and 2) to identify and characterize informative microsatellites and single nucleotide polymorphisms for haplotype analyses of the non-Mennonite MSUD Y438N alleles.

Program Nr: 1269 from the 1999 ASHG Annual Meeting

Is there a "Goldenhar" gene in 4p15.3? *H.-J. Ludecke[1], J.M. Graham, Jr.[2], S. Lindow[1], B. Horsthemke[1].* 1) Institut für Humangenetik, Universitätsklinikum, Essen, Germany; 2) Medical Genetics Birth Defects Center, Cedars-Sinai Medical Center, Los Angeles, CA.

Oculo-auriculo-vertebral (OAV) dysplasia or Goldenhar "syndrome" is characterized by hemifacial microsomia, microtia, preauricular tags, and epibulbar dermoids. Although most cases are sporadic, several families with autosomal dominant transmission of the disease with variable expression have been described, but without an association to a particular chromosomal region.

We describe a girl with multiple exostoses, hemifacial microsomia, microtia, an epibulbar dermoid cyst, a large VSD, hypoglycemia, immunodeficiency, and mild developmental delay. The patient has an apparently balanced translocation t(4;8)(p15.3;q24.1), which disrupts the *EXT1* gene on chromosome 8q24 in intron 5. Some clinical features of the OAV spectrum as well as immunodeficiency also occur in some patients with Wolf-Hirschhorn syndrome and a large deletion in 4p. In order to identify genes in 4p responsible for the OAV phenotype of our patient, we have constructed a genomic l-phage library from the patient's DNA and isolated clones for both translocation junctions. Furthermore, we constructed a PAC clone contig and identified two YAC clones covering the chromosome 4 breakpoint. This allowed us to map the breakpoint between D4S403 and D4S1601. Sequencing of the translocation junctions revealed microdeletions of 10 bp and 57 bp on chromosomes 4 and 8, respectively, flanked by short nucleotide stretches present on both normal chromosomes. Preliminary analysis of approximately 4.5 kb of DNA of the chromosome 4 breakpoint region did not reveal any potential gene sequences.

Interestingly, the homeobox gene *BAPX1* maps approximately 100 kb proximal to the translocation breakpoint. The orthologous gene of *Xenopus* is expressed in a region of the developing facial cartilage. Although our data indicate that the gene is not disrupted by the translocation, it may be affected by a position effect. The *CD38* gene in 4p15, which plays a role in T and B lymphocyte development, does not map to any of our clones and thus may not be involved in the immunodeficiency in our patient.

Program Nr: 1270 from the 1999 ASHG Annual Meeting

Mouse A9 cells containing a single human chromosome for the analysis of genomic imprinting. *S. Maegawa[1], N. Kubota[1], E. Nanba[1], J. Inoue[2], H. Kugoh[2], K. Mitsuya[2], M. Meguro[2], K. Shigenami[2], T.C. Schulz[2], M. Oshimura[2,3]*. 1) Gene Research Center, Tottori University, Yonago, Tottori, Japan; 2) Department of Molecular and Cell Genetics, School of Life Sciences, Faculty of Medicine, Tottori University, Yonago, Tottori, Japan; 3) Core Research for Evolutional Science snd Technology (CREST), Japan Science and Technology Corporation (JST), Japan.

To develop a systematic in vitro approach for the study of genomic imprinting, we generated human/mouse A9 monochromosomal hybrids. We made whole cell fusion and microcell-mediated chromosome transfer to generate A9 hybrids containing a single, intact, bsr or neo-tagged human chromosome derived from primary human fibroblasts. A9 hybrids that contained either human chromosome 1, 2, 4, 5, 6, 7, 8, 10, 11, 15, 18, 19, 20, or X were identified by fluorescence in situ hybridization and DNA analyses. The parental origin of these chromosomes was determined by DNA polymorphic analysis using microsatellite markers, and matched hybrids containing maternal and paternal chromosomes were identified for chromosomes 1, 5, 6, 10, 11, 15, 18 and 19. The parental-origin-specific expression patterns of several known imprinted gene on different human chromosomes were maintained in the A9 hybrids. This library of human monochromosomal hybrids is valuable resources for the mapping and a novel in vitro system for screening of imprinted genes and for their functional analyses. *in vitroin vivoin situ*.

Program Nr: 1271 from the 1999 ASHG Annual Meeting

A cDNA selection approach to isolate Y chromosome genes expressed in testis. *E. Makrinou[1], K. Taylor[1], M. Lovett[3], R. Del Mastro[3], Y. Edwards[2]*. 1) Biology,The Galton Laboratory, University College of London, London, England; 2) Medical Research Council, Human Biochemical Genetics Unit, The Galton Laboratory; 3) University of Texas; Southwestern Medical Centre; Dallas USA.

Statistical analysis has revealed that 2-7% of couples remain childless at the end of their reproductive life. In about half of these cases of infertility, the problem lies with the male partner. It is expected that genes expressed in the testis and involved in spermatogenesis, will occur on the mammalian Y chromosome. This view has been confirmed with the isolation of the RBM and DAZ gene families. In an effort to identify further Y-linked testis specific genes, a cDNA selection library was constructed, using adult testis cDNA, and DNA from 1,000 Y-linked cosmid clones. This library was made in collaboration with M. Lovett and R. Del Mastro at the University of Texas. Screening with vector sequencs and Y chromosome repeat sequences, has led to the elimination of 91% of the clones. 414 potential cDNA clones remain, and so far 60 have been sequenced. cDNA clones with homology to EST's and STS's on the Y chromosome have been identified. Five of these seem to represent novel members of two gene families. These genes have been localised on the Y chromosome by FISH analysis, and RT-PCRs on tissues that have been known sex determined, show that they are expressed in testis. We are in the process of screening a panel of DNA from oligo- and azoospermic patients for deletions in these genes.

Program Nr: 1272 from the 1999 ASHG Annual Meeting

Molecular characterization of carnitine palmitoyltransferase 1 deficiency in a Canadian Hutterite family. *N. ABADI[1], L. THUILLIER[1], C. PRASAD[5], L. DILLING[5], F. DEMAUGRE[1], C. PRIP-BUUS[3], A. BELBACHIR[1], M. BRIVET[4], N. KADHOM[2], J.P. BONNEFONT[1,2]*. 1) Genetic Biochemistry Unit and; 2) Unit INSERM U393, CHU Necker, PARIS; 3) Ceremod CNRS UPR 9078 MEUDON; 4) Biochemistry Unit,CHU Bicetre, FRANCE and; 5) Health Science Center, Section of Genetics and Metabolism, Winnipeg, CANADA R3AIR9.

Carnitine palmitoyltransferase (CPT) deficiencies are autosomal recessive disorders of mitochondrial long-chain fatty acid (LCFA) oxidation. While the “muscular“ and “hepato- cardio- muscular“ phenotypes of the diseases have been ascribed to a deficiency of CPT2, an enzyme of the inner mitochondrial membrane, the “hepatic“ presentation is known to result from a CPT1 deficiency. CPT1, an enzyme of the outer mitochondrial membrane that controls LCFA oxidation, has two distinct isoforms (the so-called “liver“ and “muscle“ CPT1s) encoded by two different genes. More than 10 patients with a L-CPT1 deficiency have been hitherto reported, but only one CPT1 mutation has been identified to date. We report here the resolution of the molecular defect in three L-CPT1 deficient patients belonging to an extended inbred Hutterite kindred (Haworth et al. J. Pediatr. 1992:553-557). The patients suffered from recurrent attacks of hypoketotic hypoglycemia with hepatomegaly. CPT1 activities in their fibroblasts were 10 to 15 % of controls. Sequencing of the full-length L-CPT1 cDNAs detected a homozygous G®A transition at nt 2129, predicting a Gly710®Asp substitution in all three patients. This mutation was not found in genomic DNAs from 50 controls. The substituted aminoacid is located within a stretch of 16 aminoacids highly conserved across species and CPT isoforms. This stretch has recently been suggested to be involved in the binding of carnitine to CPT2 in the rat. A functional analysis based on heterologous expression of the mutant CPT1 in yeast is under progress to study the consequences of the mutation upon the enzyme activity and the CPT1 immunoreactive protein. This analysis might contribute delineating a protein region critical for the function of this enzyme.

Program Nr: 1273 from the 1999 ASHG Annual Meeting

Trimethylaminuria is caused by mutations of the *FMO3* gene in a North American cohort. *B.R. Akerman[1], D.M. Lambert[1], H. Lemass[5], L.M.L. Chow[3], C. Greenberg[4], O.A. Mamer[2], E.P. Treacy[1]*. 1) Biochemical Genetics Unit, McGill Univ./Montreal Children's Hosp, Montreal, QC, Canada; 2) Mass Spectrometry Unit, McGill Univ., Montreal, QC, Canada; 3) Hospital for Sick Children, Toronto, ON, Canada; 4) Children's Hospital, Winnipeg, MB, Canada; 5) Mater Hospital, Dublin, Ireland.

Flavin-containing monooxygenases (FMOs) are microsomal NADPH-dependent enzymes that catalyze the oxygenation of nitrogen, sulfur, phosphorous and other heteroatom-containing chemicals, drugs and pesticides. Currently known FMOs comprise a 5-member family. In adult human liver, FMO3 constitutes the predominant form that metabolizes the above to polar readily excreted oxygenated metabolites. Trimethylaminuria (TMAuria; McKusick 136131) is an autosomal recessive condition caused by a partial or total incapacity to catalyze the N-oxygenation of the odorous compound trimethylamine (TMA), derived from dietary choline and lecithin. The result is a severe body odor and associated psychosocial conditions. This inborn error of metabolism, previously considered rare, is being increasingly detected in severe and milder presentations. We have previously shown that 4 mutations of the *FMO3* gene are causative of TMAuria in Australians (Treacy et al. 1998; Akerman et al. 1999). Of 28 individuals referred to our Unit for investigation of malodor from N. America, 10 had severe TMAuria (less than 50% of TMA oxidation) as measured by a FAB-MS assay of TMA and TMA N-oxide. We performed mutation analysis in 8 of these probands. In addition to three previously described FMO3 mutations (P153L, E305X and R492W), we detected 4 new mutations: 2 missense (A52T, (1); R387L, (2)) and one nonsense mutation (E314X). The fourth allele is apparently composed of 2 relatively common polymorphisms (K158-G308) found in the general population. Screening for known *FMO3* mutations indicates that severe TMAuria alleles are rare in a Quebec control population and thus TMAuria is predicted to be rare in this population. We conclude that one predominant mutation (P153L) and an increasing number of private mutations of different ethnic origins will account for TMAuria in North Americans.

Program Nr: 1274 from the 1999 ASHG Annual Meeting

Detection of 16 new mutations in Fabry disease. *G. Altarescu, L. Goldfarb, K-Y. Park, C. Kaneski, S. Litvak, R. Schiffmann.* NINDS, National Institutes of Health, Bethesda, MD.

Objective: Characterization of alpha-galactosidase A (AGA) gene mutations in a cohort of Fabry disease patients (n=32).Introduction: Fabry disease is an X-linked inborn error of metabolism caused by deficient activity of the lysosomal enzyme alpha-galactosidase A. Glycosphingolipids accumulate in different tissues causing multisystem disease. The main manifestations of Fabry disease include vascular damage in the heart, brain, and kidney, leading to organ failure in early adulthood. The 12-Kb gene for alpha-galactosidase A is located on chromosome Xq22.1 and consists of 7 exons varying in size from 92 to 291 base pairs. Mutations identified in the AGA gene are found throughout all exons. Most families have private mutations consisting of: large rearrangements, insertions, deletions and point mutations.Material and Methods: Blood and skin biopsy specimens were collected from 32 Caucasian patients (30 males and 2 females, 25 families). DNA was extracted using Puregene Genomic DNA Isolation Kit, Gentra Systems Inc. The cells were lysed in the presence of a DNA preservative using an anionic detergent and the genomic DNA was isolated by precipitation with alcohol 70%. Each of exons was amplified by PCR. The samples were then sequenced and the mutations identified.Results: Sixteen of the mutations detected were novel: point mutations, small insertions or deletions leading to the following amino acid changes:Met51Lys; His9X; Pro6X; Val123Met;Trp162X; Glu 250X; Gly261Asp; Lys240X; His302X; Ile 303X; Leu403X; Ser 345 X; Phe 396X (X-stop codon). Conclusions: Our investigation confirms that Fabry disease is associated with multiple mutations in the AGA gene. These findings emphasize the large variety of mutations causing Fabry disease. We have found additional 16 novel mutations to the 125 previously described AGA variants.

Program Nr: 1275 from the 1999 ASHG Annual Meeting

Biochemical and molecular investigations of nonketotic hyperglycinemia. *D.A. Applegarth[1,2], J.R. Toone[2], M.B. Coulter-Mackie[2]*. 1) Director, Biochem Diseases Lab, British Columbia Children Hosp, Vancouver, BC, Canada; 2) Department of Pediatrics, University of B.C. Vancouver, Canada.

Non ketotic hyperglycinemia (NKH) is an autosomal recessive disorder with an incidence in British Columbia, Canada of 1:63,000 live births. It is the commonest disease of amino acids in BC, after PKU, with an incidence greater than the sum of all urea cycle diseases over a 25 year period. One third of patients die in the neonatal period. Those who survive have profound neurological impairment because there is no adequate therapy. NKH is caused by a deficiency of glycine cleavage enzyme (GCE) which is a 4 protein complex (P, H, T and L proteins). L protein deficiency causes additional abnormalities in branched chain amino acid and organic acid metabolism. Identification of P, H or T protein deficiency has rarely been done since the assays require purification of the proteins of the complex. Following a diagnosis of NKH by GCE assay in liver, we have devised a screening strategy to determine which protein is deficient. Liver glycine exchange (Hirago and Kikuchi (1980) JBC 255:1166411670) without added purified H protein has no activity in patients with P or H protein deficiency and shows activity in patients with T protein deficiency. This enzymatic screening strategy is illustrated by the work-up of a patient with classical NKH presenting as a neonate and with no evidence of branched chain amino acid abnormalities. A liver biopsy showed absence of GCE activity and present glycine exchange activity. The patient was shown to be homozygous for R320H (Nanao et al (1994) Hum Gen 93: 655-658) in exon 8 of the T protein gene.

Program Nr: 1276 from the 1999 ASHG Annual Meeting

Protein sufficiency and growth in Phenylketonuria (PKU). *G.L. Arnold, C.J. Vladutiu, J. DeLuca, E. Blakely.*
Division of Pediatric Genetics, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA.

Children with PKU often exhibit sub-optimal growth. This has been attributed to various factors, including inadequate protein intake or poor utilization of L-amino acid protein supplements. We reviewed the records of 39 children ages 3-20 years with classic, early and continuously treated PKU in order to study the relationship between protein sufficiency and growth. Variables included plasma prealbumin, age, and height percentile for age on the National Center for Health Statistics growth curves. Plasma prealbumin values were available for the most recent three or four annual evaluations. For most children, variability between these annual values was less than 10%. For the two children with more variability in prealbumin measurements, the mean value of three annual measurements was used.

Mean age was 8.9 years, mean height was at the 45.8%ile, and mean plasma prealbumin was 20.6 mg/dl. We identified a significant correlation between height percentile and prealbumin, such that children with lower plasma prealbumin tended to be shorter ($r=0.387$, $p<0.025$). There was also a strong correlation between age and prealbumin, with older children having higher prealbumin ($p<0.01$). However, there was no correlation between height percentile and age. Children whose height was less than the 25%ile had lower prealbumin than those with height greater than the 75%ile (17.25 vs 23.0 mg/dl, $p<0.01$). Children with prealbumin <20 mg/dl were younger than those with higher prealbumin (6.6 years vs. 10.6 years, $p<0.001$) and were significantly shorter (30%ile vs. 57%ile,$p<0.004$). These data identify a relationship between growth and plasma prealbumin in PKU, and suggest protein insufficiency plays a role in poor growth in children with PKU.

Program Nr: 1277 from the 1999 ASHG Annual Meeting

Pendred syndrome : Phenotypic heterogeneity in two families carrying the same PDS missense mutation. *H. Ayadi[1], S. Masmoudi[1], I. Charfeddine[2], M. Hmani[1], M. Grati[3], A. Ghorbel[2], A. Boulila-Elgaed[1], J.P. Hardelin[3], M. Drira[2]*. 1) Dept Immunology, Fac de Medecine de Sfax, Sfax, Tunisia; 2) Service d'ORL, CHU H. Bourguiba, 3018 Sfax, Tunisie; 3) Unit de gntique des dficits sensoriels, Institut Pasteur, 25, rue du Dr. Roux, 75725 Paris 15, France.

Pendred syndrome is a recessively inherited disease which is classically defined as the association of congenital sensorineural hearing loss, thyroid goitre and positive perchlorate discharge test. This disease has recently been shown to be caused by mutations in the PDS gene, which encodes an anion transporter called pendrin. Molecular analysis of the PDS gene was performed in two consanguineous large families from Southern Tunisia comprising a total of 23 individuals affected with profound congenital deafness; the same missense mutation, L445W, was identified in all affected individuals. Widened vestibular aqueducts were found in all the patients who underwent CT scan exploration of the inner ear. In contrast, goitre was present only in 11 affected individuals, who interestingly had a normal perchlorate discharge test whenever performed. Therefore, the present results further question the sensitivity of the perchlorate test for the diagnosis of Pendred syndrome and would support the use of a molecular analysis of the PDS gene in the assessment of individuals with severe to profound congenital hearing loss associated with inner ear morphological anomaly, even in the absence of a thyroid goitre.

Program Nr: 1278 from the 1999 ASHG Annual Meeting

SEVERE HYPOGLYCEMIA AS A PRESENTING SYMPTOM OF CARBOHYDRATE-DEFICIENT GLYCOPROTEIN SYNDROME. *D. Babovic-Vuksanovic[1], M.C. Patterson[1], W.F. Schwenk[1], J.F. O'Brien[1], J. Vockley[1], H.H. Freeze[2], D.P. Mehta[2], V.V. Michels[1]*. 1) Dept Medical Genetics, Dept Pediatric and Adolescent Medicine, Dept Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN; 2) The Burnham Institute, La Jolla, CA.

Carbohydrate-deficient glycoprotein syndrome (CDGS) Type Ib is a newly recognized form of CDGS caused by deficiency of phosphomannose isomerase (PMI). The phenotype in affected patients is fundamentally different from the CDGS Type Ia. There is no cognitive or neurologic impairment, but marked gastrointestinal and liver symptomatology. We describe clinical, biochemical and molecular findings in a 2 years, 6 month old girl with PMI. Our patient presented with severe and persistent hypoglycemia, and subsequently developed protein-losing enteropathy, liver disease and coagulopathy. Six months of therapy with mannose supplementation resulted in clinical improvement and partial correction of biochemical abnormalities. It is important to increase awareness of this disease since early diagnosis and therapy may be beneficial. CDGS Type Ib should be considered in the differential diagnosis of patients with unexplained hypoglycemia, especially in presence of other symptoms such as chronic diarrhea, cyclic vomiting, liver disease or coagulopathy.

Program Nr: 1279 from the 1999 ASHG Annual Meeting

Facioscapulohumeral Muscular Dystrophy Myoblasts Have Increased Susceptibility to Oxidative Stress. *K.A. Barrett[1,2], J.D. Forrester[1], R. Tawil[1], R.C. Griggs[1], D.A. Figlewicz[1,2].* 1) Department of Neurology, University of Rochester School of Medicine & Dentistry , Rochester, NY; 2) Department of Neurobiology & Anatomy, University of Rochester School of Medicine & Dentistry, Rochester, NY.

Facioscapulohumeral Muscular Dystrophy (FSHD), the third most common muscular dystrophy, with an estimated prevalence of 1:20,000, is inherited as an autosomal dominant trait. Although no specific gene defect has been identified, FSHD has been associated with a deletion in a repeat region on chromosome 4q35. In culture, FSHD myoblasts display general signs of necrosis (cytoplasmic vacuoles and swelling) and differentiate into disorganized, vacuolated myotubules. In an effort to better understand this phenotype, the current study exposed FSHD myoblasts, myotubes and normal control cells to paraquat, a generator of superoxide radicals, and staurosporine, a protein kinase C inhibitor. Cells were obtained from muscle biopsies and grown to approximately 60% confluence in high serum-containing growth media. Normal growth media was removed from the myoblast cultures and replaced with media containing varying concentrations of paraquat or staurosporine for 24 and 72 hours. Survival for each concentration of toxin was expressed as a fraction of growth in sister wells containing normal growth media. Another set of cells was differentiated into myotubules and processed as described above. Results demonstrate a statistically significant increased susceptibility of FSHD myoblasts to paraquat compared to normal control myoblasts, whereas no such difference was observed for staurosporine exposure. FSHD myotubules and normal myotubules possess an equivalent vulnerability to both toxins. These results are in stark contrast to dystrophin lacking cells. In a similar study, Rando et al (1997) found that dystrophin lacking myotubules, but not myoblasts, were differentially susceptible to oxidative stress. The current work indicates a defect early in myogenesis in FSHD, perhaps in cell cycle regulation. Supported in part by grants from the MDA-USA; New York State Dept of Education; and the Wayne C. Gorell, Jr. Laboratory of the University of Rochester.

Program Nr: 1280 from the 1999 ASHG Annual Meeting

A simple PCR based assay allows detection of a common mutation, IVS8-1G>C, in DHCR7 in Smith-Lemli-Opitz Syndrome patients. *K.P. Battaile[1], C.L. Maslen[2], F.D. Porter[3], R.D. Steiner[1]*. 1) Pediatrics and Molecular & Medical Genetics, Oregon Health Sciences University, Portland, OR; 2) Department of Medicine, Oregon Health Sciences University, Portland, OR; 3) Heritable Disorders Branch, NICHHD, Bethesda, MD.

Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive condition resulting from a deficiency of the enzyme 7-dehydrocholesterol-delta7-reductase. SLOS is a clinically heterogeneous syndrome with symptoms ranging from severe congenital anomalies to mild mental retardation. We and other groups have previously reported that mutations in DHCR7 cause SLOS. We have developed a novel PCR based assay to detect the common mutation in SLOS, IVS8-1G>C, which is a G to C transversion in the splice acceptor site 5 to exon 9. This mutation results in the elimination of a ScrF1 restriction and the inclusion of 134 bp of intron in the cDNA, presumably due to the use of an alternate upstream splice site. Our assay involves amplification of a 129 bp region of the genomic DNA then digestion of the PCR product with ScrF1 followed by visualization of products by agarose gel electrophoresis.

Using this novel assay we rapidly screened genomic DNA from 18 patients and 43 controls. We found heterozygosity for this mutation in 10, and homozygosity in 1 of 18 unrelated patients and none of the controls. The high incidence of this mutation in SLOS in conjunction with the simplicity and reliability of this PCR based assay makes this a convenient test to aid in patient diagnosis and carrier detection, and opens up the possibility of prenatal molecular genetic diagnostic testing.

Program Nr: 1281 from the 1999 ASHG Annual Meeting

EFFECTS OF WILD-TYPE OR MUTANT HUNTINGTIN IN STRIATAL CELLS. *J.H Bauer*[*2], D. Rigamonti*[*1], C. De-Fraja[1], L. Conti[1], S. Sipione[1], C. Sciorati[3], E. Clementi[3], A. Hackam[4], M.R. Hayden[4], Y. Li[2], J.K. Cooper[5], C.A. Ross[5], C. Vincenz[2], E. Cattaneo[1]*. 1) Institute of Pharmacological Sciences, University of Milano, Via Balzaretti 9, 20133 Milano, Italy; 2) Dept. of Pathology, University of Michigan, Ann Arbor, MI, U.S.A; 3) Di.Bi.T. San Raffaele Hospital, Milano, Italy; 4) University of British Columbia, Vancouver, British Columbia, Canada; 5) Dept. of Psychiatry, Johns Hopkins University, Baltimore, U.S.A.

Huntington's Disease (HD) is due to a defect in a gene called IT15 characterized by the presence of an unstable CAG repeat encoding for a polyglutamine sequence. Despite ubiquitous expression of the normal and mutant Huntingtin (Htt) protein, HD results in a selective neuronal loss prominent in the basal ganglia and in the cerebral cortex. The mechanism of cell death and the functions of either the normal or the mutant proteins are still unknown. Cellular models of HD would greatly assist this analyses. DARPP-32 positive striatal derived ST14A cells (Cattaneo and Conti, Journal Neurosci. Res., 1998) have been stably transfected with various constructs containing different portions of the Htt protein in the wild-type or in the mutant form. We found that wild-type Htt acts by protecting cells from a variety of apoptotic stimuli. Protection takes place upstream of caspase 3. The full length protein also modulates the toxicity of the poly-Q expansion. Cells expressing full length mutant protein are susceptible to fewer death stimuli than cells expressing truncated mutant Huntingtin *co-first authors (supported by the Huntington's Disease Society of America, the Hereditary Disease Foundation, Telethon (Italy #E840), C.N.R. (Italy, #98.01050.CT04) to E.C., NIH-ROI E50811 and DAMD 17-96-1-6085 to C.V. and NIH NS16375 to C.A.R.).

Program Nr: 1282 from the 1999 ASHG Annual Meeting

Normal size cubilin in a patient with megaloblastic anemia 1. *E.V. Bawle[1], S.K. Moestrup[2].* 1) Genetic Metab/Disorders, Children's Hosp Michigan, Detroit, MI; 2) Dept.of Medical Biochemistry,Univ of Aarhus,Aarhus,Denmark.

Megaloblastic anemia 1(MGA1,OMIM 261100)is an autosomal recessive disorder characterized by juvenile megaloblastic anemia, neurological symptoms and benign proteinuria. It is caused by selective malabsorption of intrinsic factor(IF)-vitamin B_{12}complex and is also known as Imerslund-Gräsbeck's disease. Recently, circumstantial evidence for a defective IF-B_{12}intestinal receptor named cubilin as the basis for this condition has been published. Moreover,in 17 Finnish families with MGA1,mutations in CUBN,the gene encoding cubilin,have been reported,indicating CUBN to be the disease causing gene. A missense mutation with normal size cubilin was reported in the majority of those families. We report a MGA1 patient of German-Scottish ancestry with normal size cubilin. Her symptoms began at the age of 8 years with weakness in lower extremities,ataxia and urinary incontinence. Her fullscale IQ was in the borderline range(72) and she was having difficulty in keeping up with 3rd grade work. She showed proximal muscle weakness and loss of sense of vibration in the lower extremities. An EMG & nerve conduction in the legs showed demyelinating motor and sensory neuropathy. Brain MRI showed mild cortical atrophy. Her hemoglobin was normal but there was macrocytosis of the RBCs. Methylmalonic & methylcitric aciduria were found by organic acid screening. Serum vitamin B_{12}level was <60 pg/ml(normal 187-1059)with normal folate level.IF blocking antibodies were not detected in the serum. Urine had 10-20 RBCS/hpf,132 mg/dl protein,and protein/creatinine of 1.A Schilling test with & without IF showed B_{12}urinary excretion of 0.4%& 0.2%(average normal 20%)confirming IF-B_{12}receptor defect. Her neuropathy disappeared and school work improved with a B_{12}injection every 10 days. An analysis of a freshly obtained immediately frozen urine by SDS-PAGE showed the same protein profile of proteinuria as other MGA1 patients. The size of the urine cubilin estimated by western blotting was comparable to that of normal urine and IF-B_{12}affinity purified cubilin thus excluding a major deletion in CUBN. Our patient either has a functional defect in the cubilin or there is genetic heterogeneity.

Program Nr: 1283 from the 1999 ASHG Annual Meeting

Mutational analysis, carrier detection, and parental origin of mutation studies in 39 families affected with Hunter syndrome. *A. Bellows, J. Thompson.* Department of Human Genetics, University of Alabama at Birmingham, Birmingham, AL.

Hunter syndrome (Mucopolysaccharidosis type II) is an X-linked disorder resulting from a deficiency of the lysosomal sulfatase, iduronate-2-sulfatase (IDS). Two forms of Hunter syndrome, mild and severe, are recognized representing the two ends of a wide spectrum of clinical manifestations. The clinical variability seen among Hunter patients is believed to be due to genetic heterogeneity within this gene. The IDS gene maps to Xq28, spans 24kb, and contains 9 exons and 8 introns. In the present investigation, 39 patients were enzymatically diagnosed with Hunter syndrome by the demonstration of IDS deficiency. To further delineate the IDS gene defects, total RNA and genomic DNA were extracted from peripheral leukocytes or cultured fibroblasts from the patients and their at-risk female relatives. Mutation detection was performed by sequencing of cDNA fragments. Mutation confirmation and heterozygote analysis was done by restriction enzyme digestion or sequencing of the appropriate exon from genomic DNA. Among the 39 mutant alleles identified, there were 24 missense mutations, 1 nonsense mutations, 8 mutations affecting splicing, 5 small deletions or insertions, and 1 complete deletion of the IDS gene. Fifteen of the mutations were novel and unique, however, 1 of the new mutations was found in 2 unrelated patients. Five mutations (R88C, S333L, S349R, R468Q, and 1122C®T) were found repeatedly in this study, indicating that these codons are hotspots for mutation. The majority of the mutations (64%) were found in exons 3, 8, and 9. Of the 34 mothers available for study, 29 (85%) were identified as carriers. In the patient with the complete deletion, haplotype analysis revealed haploinsufficiency in both the proband's mother and sister, thus confirming their heterozygote status. Haplotype analysis was used to determine the parental origin of mutation. In 10 families in which multigenerational members were available, 5 families were found to have a paternal origin of mutation and 5 had a maternal origin. The present investigation further emphasizes the genetic heterogeneity of the IDS gene.

Program Nr: 1284 from the 1999 ASHG Annual Meeting

Cloning and expression of guinea pig lysosomal a-**mannosidase and characterization of a mutation causing** a-**mannosidosis in a guinea pig colony.** *T. Berg, J.J. Hopwood.* Chemical Pathology, Women's and Children's Hospital, Adelaide, South Australia.

Lysosomal a-mannosidase is an exoglycosidase involved in the degradation of glycoproteins. Deficiency of this enzyme leads to the lysosomal storage disorder a-mannosidosis. The inherited form of a-mannosidosis has been identified in humans, cattle, cats, and recently, also in guinea pigs. At the cellular level the disease is characterized by lysosomal accumulation of mannose-containing oligosaccharides. Clinically, affected individuals express progressive mental deterioration in addition to a number of non-neurological symptoms involving the visceral organs and the blood and the skeletal systems. Affected cattle and cats usually die before they reach 6 months of age. The phenotype in guinea pigs is significantly milder as affected animals survive into adulthood (>1 year). Hence it resembles the human form where most patients survive into adulthood. In order to characterize the guinea pig model at the molecular level, we used a PCR-based strategy to isolate and sequence the full length a-mannosidase cDNA from guinea pigs. The cDNA sequence contained an open reading frame encoding 1007 amino acids with 79% sequence identity to the human a-mannosidase sequence. Transient expression of the cDNA in COS-7 cells gave a 7 fold elevation in the a-mannosidase level. Sequencing of the a-mannosidase cDNA from an affected animal revealed a mutation causing substitution of a conserved arginine with tryptophan (R227W). Further, PCR-based screening demonstrated that the R227W mutation was uniquely associated with a-mannosidosis in the guinea pig colony and thus supporting the notion that the R227W mutation is causing disease. Mass spectrometry revealed that the storage products in guinea pigs cells and urine contained only single GlcNAc residues at their reducing ends. Hence, the stored oligosaccharides in guinea pigs are, in contrast to the other animal models, identical to the storage products in human patients. This work will form the basis for development and evaluation of therapeutic strategies for neuronal storage diseases using guinea pigs as a convenient model.

Program Nr: 1285 from the 1999 ASHG Annual Meeting

Prolidase deficiency: Clinical and biochemical characterization of the disease and prenatal diagnosis in a genetic isolate in Israel. *Z.U. Borochowitz[1], T.C. Falik-Zaccai[1], M. Jaffe[2], P. Savoie[3], N. Abeling[4], A.H. van Gennip[4], P. Hechtman[3].* 1) Simon Winter Inst Medical Gen, Bnai-Zion Med. Ctr. Haifa, Israel; 2) Dep. Pediatrics, Bnai Zion Med. Cent. Haifa Israel; 3) Dep. Clin Biochem, Montreal Child Hosp, Canada; 4) AMC.Univ of Amsterdam, The Netherlands.

Prolidase deficiency (PD) is a rare autosomal recessive metabolic disorder. The clinical phenotype varies among affected individuals from no manifestation to severe progressive skin lesions, severe infections and impaired cognitive development. We have studies 6 patients from a large, highly consanguineous, Arabic Moslem kindred and report here the first prenatal diagnosis for PD. Three of the 6 patients presented within the first year of life with fulminant sepsis. The 3 others presented with various degrees of developmental delay, failure to thrive, facial dysmorphism and eczema. One patient developed clinical and immunologic abnormalities consistent with systemic lupus erythematosus. Using Bidimentional TLC and amino acid analyzer on urine samples before and after hydrolysis has revealed large amounts of iminopeptited in the urine of 5 of the 6 patients. Prolidase activity was absent in fibroblasts of 2 patients. Following the biochemical analysis prenatal diagnosis was offered to couples at risk within the large kindred. Amniocytes were cultured and studied for prolidase specific activity (uMoles Proline/h/mgProt) which was found to be markedly reduced. The fetus was predicated to be affected but parents have decided to continue the pregnancy. Diagnosis of PD was confirmed after birth studying prolidase activity in cultured fibroblasts of foreskin (1.42 +/-0.54) compared to healthy control (20.46 +/-0.99) and resembling a known unrelated PD cell line (0.23+/-0.26) and his affected sister (0.20 +/-0.16). At the age of 4 month this baby is well developed for his age, slightly dysmorphic and suffers severe episodes of bronchitis and diarrhea. PD is highly prevalent in this Arabic village. Prenatal diagnosis to couples at risk of a non treatable metabolic disorder of such highly variable expression is complicated and should be considered following comprehensive genetic counseling.

Program Nr: 1286 from the 1999 ASHG Annual Meeting

Analysis of PON1 Status Requires Both Genotype and Phenotype. *V.H Brophy, G.P. Jarvik, R.J. Richter, L.S. Rozek, J.J. Lum, C.E. Furlong.* Medicine, Div. of Med. Genetics, University of Washington, Seattle, WA.

Paraoxonase (PON1), an HDL associated enzyme, is believed to protect against atherosclerosis by metabolizing oxidized lipids. PON1 also hydrolyzes the bioactive oxon forms of organophosphate pesticides such as parathion, diazinon, and chlorpyrifos. Two polymorphisms have been identified in the coding sequence of human PON1: met/leu at position 55, arg/gln at 192. While $PON1_{192}$ affects the relative rates of hydrolysis for different substrates, $PON1_{55}$ does not. Previous studies found that $PON1_{M55}$ individuals have lower levels of PON1 mRNA and activity. In this study, we examined the relationships between genotype and PON1 levels. We found considerable overlap in activity level for diazoxon and paraoxon hydrolysis among the $PON1_{55}$ genotypes. Of 274 individuals whose $PON1_{192}$ status was detected by two-dimensional enzyme analysis, 129 (46%) were $PON1_{QQ192}$. Of these, 31 (24%) were $PON1_{LL55}$, 69 (54%) $PON1_{L/M55}$ and 29 (23%) were $PON1_{MM55}$. Analysis of $PON1_{QQ192}$ individuals showed that while the average PON1 diazoxonase activity was 13395 U/L (±3409.8) for $PON1_{LL55}$ and 9120.3 U/L (±2804) for $PON1_{MM55}$, a given $PON1_{MM55}$ could have more than twice the activity of a $PON1_{LL55}$ individual. The $PON1_{192}$ and $PON1_{55}$ polymorphisms account for 74.5% of the interindividual variation in PON1 paraoxonase and only 33.9% in the diazoxonase activity. After adjustment for $PON1_{192}$ genotype the $PON1_{55}$ polymorphism accounts for only 17.3% of the paraoxonase and 13.4% of the diazoxonase residual activity.

$PON1_{R192}$ has a high turnover number for paraoxon than $PON1_{Q192}$ but lower for diazoxon and soman. Seven previous studies have found that the R_{192} allele raises the risk of cardiovascular disease while five studies have found no correlation. The studies, however, examined the genotype of PON1 only, not expression levels. We found expression level varies widely among individuals and within a genotype. Diazoxonase activity varied 5-fold among $PON1_{QQ192}$, 6-fold among $PON1_{Q/R192}$, and 6-fold among $PON1_{RR192}$. Quantification of PON1 enzyme activities may improve the predictive power of PON1 genotype as a risk factor in cardiovascular studies.

Program Nr: 1287 from the 1999 ASHG Annual Meeting

Enzymatic and molecular pre and postnatal diagnosis of ceroid lipofuscinoses in France. *C. Caillaud[1], J. Manicom[1], J.P. Puech[1], P. Lobel[2], L. Poenaru[1]*. 1) Dept Genetique, CHU Cochin, Paris, France; 2) Center for Advanced Biotechnology and Medicine, Piscataway, New Jersey 08854, USA.

The neuronal ceroid lipofuscinoses (NCL) are a group of inherited neurodegenerative disorders characterized by the accumulation of intralysosomal autofluorescent lipopigments, particularly in neurons. Their main clinical signs are psychomotor retardation, impaired vision and seizures. Four NCL forms have been described according to clinical and morphological features : infantile (INCL), late-infantile (LINCL), juvenile (JNCL) and adult (ANCL). Infantile NCL is caused by defects in the gene CLN1, which encodes a palmitoyl protein thioesterase. Four genes are involved in LINCL : CLN2, encoding a pepstatin-insensitive peptidase, CLN5, encoding a transmembrane protein of unknown function, and two uncloned genes, CLN6 and CLN7. In JNCL, the product of the defective gene, CLN3, is a lysosomal transmembrane protein. The gene responsible for the adult form, CLN4, remains to be identified. Patients with LINCL, the most frequent clinical form in France, were tested for the pepstatin-insensitive peptidase (or pepinase) activity. Only half of the patients exhibited a lack of pepinase activity. These patients were tested for CLN2 mutations, using PCR and automatic sequencing. Two previously described mutations were found in one patient each : an intronic G>C transversion at the 3 splice junction of intron 5 and an Arg208Stop in exon 6. Novel mutations were characterized in the other patients : two point mutations, Gln509Stop in exon 12 and Ser153Pro in exon 5, one 11-bp deletion in exon 13 and a A>G transition at the 3' splice junction of intron 8. A prenatal diagnosis was performed for a couple at risk for the infantile form of ceroid lipofuscinosis. The enzymatic and molecular methods were in accordance, revealing a pepinase deficiency and the presence of both parental mutations. This prenatal diagnosis was the first done by using such techniques in France. Our results demonstrates 1-the usefulness of a new enzymatic method for pre and postnatal diagnosis of LINCL patients, 2-the heterogeneity of the CLN2 mutations, 3-the diversity of the loci involved in the pathogenesis of LINCL.

Program Nr: 1288 from the 1999 ASHG Annual Meeting

A retroviral gene trap insertion at the mouse *rab9* locus. *J.P. Cannon[1], S.M. Colicos[1], S.R. Pfeffer[2], J.W Belmont[1].* 1) Dept Mol & Human Genetics, Baylor Col Med, Houston, TX; 2) Dept Biochem, Stanford Univ School of Med, Stanford, CA.

Rab9 is a member of a large family of GTPases involved in membrane system-specific vesicular trafficking within cells. Dominant-negative mutant analysis has implicated Rab9 in endosome to trans-Golgi trafficking. Rab9 is required for recycling of the mannose-6-phosphate receptor (MPR) from endosome to trans-Golgi and, therefore, plays an essential role in lysosomal enzyme targeting. We have identified a retroviral gene trap insertion at the mouse *rab9* locus, downstream of a single *rab9* coding exon. Expression of the proviral bgeo gene, presumably directed by signals from the endogenous *rab9* locus, was localized to the heart, liver, branchial arches, and limb buds at embryonic day 11.5. bgeo expression in the adult was observed in the brain, liver, myocardium, and autonomic ganglia that innervate the heart. Northern blot hybridization detected *rab9* transcripts in several adult mouse tissues, with highest expression found in the adult liver. Mapping using an interspecific backcross panel placed the mouse *rab9* locus on the distal X chromosome, between the markers *DXMit149* and *DXMit122*. The *rab9*^{bgeo1} allele is apparently subject to X inactivation based on a varietgated pattern of expression in female heterozygotes. Western blot analysis of liver protein from hemizygous mutant males and homozygous mutant females showed that the *rab9*^{bgeo1} gene trap allele is hypomorphic, producing approximately 10% percent of wildtype levels of protein. *rab9*^{bgeo1} mutant animals are fertile and present no overt phenotype by six months of age. In order to better understand the role of the residual protein function, we have developed a null allele by targeted recombination in ES cells. High level male chimeras have been obtained indicating that *rab9*^{null} is not a cell autonomous lethal. Null fibroblast cell lines are being developed for studies of lysosomal enzyme transport and MPR recycling.

Program Nr: 1289 from the 1999 ASHG Annual Meeting

Respiratory-Chain Enzyme Activities in Muscle from HSP Patients. *R. Carrozzo[1], F. Piemonte[1], C. Casali[2], G. Tozzi[1], C. Patrono[1], A. Tessa[1], E. Bertini[1], F.M. Santorelli[1].* 1) Molecular Medicine Unit, Bambino Gesu Hosp, Rome, Italy; 2) Inst of Neurology, La Sapienza University, Rome, Italy.

Hereditary spastic paraplegia (HSP) is a heterogeneous group of disorders characterized by progressive weakness and spasticity of the lower limbs due to degeneration of corticospinal axons. HSP is classified according to both the mode of inheritance (autosomal dominant, recessive, and X-linked) and whether progressive spasticity occurs in isolation (pure forms) or with other neurologic abnormalities (complicated HSP), including optic neuropathy, retinopathy, dementia, ataxia, and deafness. It has been shown that the first HSP gene (SPG7) encodes for a protein (paraplegin) which localizes to mitochondria. Homozygous SPG7 mutations produce typical morphological signs of mitochondrial oxidative phoshorylation (OXPHOS) defects. This suggests an altered OXPHOS mechanism for neurodegeneration in HSP-type disorders. We analyzed respiratory chain enzymes in muscle from HSP patients, negative for paraplegin alterations. Morphological examination of the biopsied muscle (deltoid in four cases, biceps femoralis in two) showed reduced histochemical stain for cytochrome c oxidase (COX) and subsarcolemmal accumulation with succinate dehydrogenase (SDH) stain. Biochemically, we found a statistically sigificant COX reduction (1.6+/-0.4; controls 2.3+/-0.1; $p<0.05$) when values were corrected for the activity of citrate synthase. Complex I activity was also reduced in two patients (40%; and 60%; controls). SDH activity was normal. Interestingly, all the affected enzymatic activities are under a double genetic control, the nDNA and mtDNA. This might suggest a still unclear role for mtDNA sequences in spite of lack of maternal transmission. The role of the mitochondrial antioxidant system in HSP patients is also being investigated. Our data propose the methodical use of a muscle biopsy when examining HSP patients to recognize mitochondrial alterations.

Program Nr: 1290 from the 1999 ASHG Annual Meeting

Molecular analysis of Japanese patients with homocystinuria due to cystathionine beta-synthase deficiency. *S. Chen, M. Ito, T. Saijo, E. Naito, I. Yokota, J. Matsuda, Y. Kuroda.* Department of Pediatrics, School of Medicine, University of Tokushima, Japan.

Homocystinuria due to cystathionine b-synthase (CBS) deficiency is an inborn error of sulfur-amino acid metabolism. More than ninety mutations of the CBS gene in patients with homocystinuria have been reported worldwide, however, the molecular basis of this condition in Japanese is not clear. In this study, we identified eight mutations in six Japanese homocystinuric patients from five different families. The mutations detected included five novel mutations (A194G, G775A, A1321T, exon 9 skipping and 1590-1593 GTTC deletion) and three previously reported mutations (G346A, G430A and exon 12/exons11 and 12 skipping), however, the cause for exon 12/exons 11 and 12 skipping was different from the previous reports. When expressed in Escherichia coli cells, all of the mutant proteins exhibited decreased catalytic activity in vitro, confirming that all of the mutations were pathogenic. Compared with the molecular characteristics of other ethic groups, this study found that exon skipping, nonsense mutation and frame shift mutations in CBS gene were relatively common in Japanese homocystinuric patients.

Program Nr: 1291 from the 1999 ASHG Annual Meeting

Increased elastolysis in Cutis Marmorata Telangiectasia Congenita (CMTC). *D. Chitayat[1], A. Hinek[2], M. Beghetti[3], H.. Tresurer[2], M.M. Silver[4], D. Nykanen[3]*. 1) Division of Clinical Genetics; 2) Cardiovascular research; 3) Division of Cardiology; 4) Department of Pathology. Department of Pediatrics, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada.

CMTC is a systemic condition characterized by a reticular vascular skin lesions present from birth. The skin lesions are often extensive and associated with ulcerations and other complications including hemihypertrophy or hemiatrophy, aplasia cutis congenita, intracerebral hemorrhage, glaucoma, hypothyroidism, pulmonary hypertension and developmental and growth delay, seizures, macrocephaly and terminal transverse defects of the limbs. We report a child with CMTC who was born with extensive generalized phlebectasia and nevus vascularis reticularis and developed skin ulcers, intracerebral hemorrhage, retinal detachments, hypothyroidism and pulmonary hypertension. He died at the age of 20 months. Autopsy confirmed multiorgan telangiectasia, ectatic capillary and venous proliferation and severe medial thickening of the small pulmonary arteries. Further studies revealed that his fibroblast culture did not contain any elastic fibers but synthesized normal amounts of tropoelastin, formed normal microfibrillar scaffolds and produced abundant collagen type I and fibronectin. The patients fibroblasts serine elastase(s) activity was twice as high as in controls. Moreover, while incubation of the CMTC fibroblasts with 0.4 M copper sulfate significantly increased their elastolytic activity, addition of 0.4 M calcium chloride or 1-antitrypsin, but not phenantrolin and C-64 (inhibitors of metalo- and serine- proteases, respectively) reduced their elastolytic activity to normal levels. We therefore postulate that the elastolytic activity of the serine protease(s) stimulated by an increased tissue concentration of copper can result in a process which leads to the connective tissue and vascular disorders observed in our patient with CMTC.

Program Nr: 1292 from the 1999 ASHG Annual Meeting

Expression and characterization of normal and mutant human propionyl-CoA carboxylase in *E.coli*. *M. Chloupkova[1], K. Ravn[2], M. Schwarz[2], J.P. Kraus[1]*. 1) Dept Pediatrics, C-233, University of Colorado School of Medicine, Denver, CO; 2) Dept Clin Genet, Rigshospitalet, Copenhagen, Denmark.

Propionic acidemia is an autosomal recessive disorder caused by a deficiency of propionyl-CoA carboxylase (PCC). Human PCC, a mitochondrial matrix enzyme, is composed of nonidentical a and b subunits encoded by the nuclear encoded PCCA and PCCB genes, respectively. We have previously reported on successful expression of human PCC in *E.coli*. The a and b PCC cDNAs were subcloned, without their leader sequences, into a plasmid with two different promoters. Assembly of active PCC necessitated a coexpression of GroES and GroEL chaperones. We have since optimized the expression and purification of recombinant PCC to yield ~3mg/l of culture of homogeneous, fully assembled active enzyme. The kinetic properties of the recombinant PCC are nearly identical to those for PCC purified from human liver. The size of the native enzyme is consistent with an a_6b_6 organization. The relative simplicity and gentle nature of the purification procedure is ideally suited for the purification of selected mutant PCC enzymes.

Almost 60 mutations have been reported in the PCCA and PCCB genes, however, only the T428I mutation in the b subunit has been expressed and partially characterized. Exon 15 in the b subunit is one of the two sites where a number of mutations have been identified in propionic acidemia patients. The 513insP has been found at an unusually high carrier frequency of 1:17 in the Inuit population of Greenland. We have introduced this mutation into our expression construct and expressed the mutant protein in *E.coli*. The insertion of a proline completely inactivated the enzyme. Western blot analysis revealed normal amount of a and b subunits suggesting that the observed lack of activity is not due to failure to express the protein or due to the instability of the mutant subunits. We are currently analyzing additional mutations found in exon 15 in bPCC.

Program Nr: 1293 from the 1999 ASHG Annual Meeting

Glycine cleavage enzyme complex (GCE): Expression and characterization of the hydrogen-carrier (H) protein component. *F.M. Choy[1], L. Sharp[1], D.A. Applegarth[2].* 1) Dept Biol, Univ Victoria, B.C., Canada; 2) Dept Pediatrics, Univ British Columbia, Vancouver,Canada.

The H protein is a lipoic acid-containing protein and one of the 4 essential components (H, L, P, & T proteins) of the GCE, the major catabolic pathway of glycine. Defects in these protiens result in reduced GCE activity and constitute the biochemical basis of non-ketotic hyperglycinemia (NKH). Most NHK patients with the neonatal phenotype have a defect in the P protein. GCE activity assay for the biochemical diagnosis of NKH from P protein defects requires the presence of purified H protein in excess. Chicken liver is usually used as the source because it is available commercially and H protein is expressed in the liver (besides kidney & brain). However, the purification is time consuming and the yield is low. We have isolated the full length cDNA of H protein from the rabbit by reverse transcription of liver mRNA. It is comprised of 2 regions that encodes a 48-amino-acid mitochondria target sequence and a 125-amino acid residue that constitutes the mature matrix protein. There is a 73 and 98.4% sequence homology in these regions to human, versus a 43.6 and 84.5% homology in the same regions between chicken and human. Our data demonstrate that the mitochondria target and mature matrix protein regions in the H protein gene may have evolved differently. Gene diversification is noted in the former which reflects the species specificity. However, the later is very well conserved and suggests a very similar structure-function of the mature protein among these species in evolution. The mature H protein cDNA was cloned in-frame 3' to the *Saccharomyces cerevisiae* a-secretion signal in pPICZaA vector and transformed into *Pichia pastoris* KM71 strain using electroporation. Aliqots were taken from the culture medium of *P. pastoris* following methanol induction from 0 to 96 hr. The apo-H protein was highly expressed in the secreted form after 24 hr of induction and was maintained up to 96 hr. The production of sufficient amounts of recombinant H protein will facilitate the biochemical characterization of the GCE and its implications in NKH.

Program Nr: 1294 from the 1999 ASHG Annual Meeting

Why cells die in inherited neuronal degenerations (INDs): exponential death kinetics exclude cumulative damage, identify a constant or decreasing death risk, and suggest a mutant steady state model. *G.A. Clarke[1,2], R.A. Collins[2], C.J. Lumsden[3], R.R. McInnes[1,2,3].* 1) Program in Devel. Biol., Hospital for Sick Children, Toronto; 2) Dept. Molec. & Med. Genet., U of Toronto; 3) Institute. Med. Sci., U of Toronto, Canada.

INDs are generally characterized by a delay - varying from months to decades - in the onset of clinical symptoms. This presymptomatic interval has been assumed to reflect age-dependent cumulative damage. For example, cell death in INDs such as Alzheimer's disease has been suggested to result from an excess O_2 radicals which overwhelm protective mechanisms, damage macromolecules, and cumulatively disrupt metabolism (e.g. Coyle and Puttfarken, Science, 262:689, 1993; Cassarino and Bennett, Brain Res. Rev. 29:1, 1999). The cumulative damage hypothesis predicts that the probability of cell death will increase over time. To test this hypothesis, we used regression analysis to determine whether the kinetics of neuronal death in 11 animal models of inherited retinal degeneration and in patients with Parkinson's disease could be fit to mathematical models in which the risk of cell death either increased, decreased, or remained constant with age. Our analyses clearly demonstrate that the temporal patterns of cell death in these diseases are best explained by models in which the risk of death remains constant or decreases exponentially with age. Thus, the probability of neuronal death is not increased by cumulative damage (if such damage occurs), and the time of death of any neuron is random. The role of mutation is therefore to increase the probability of cell death; different mutations increase the probability to different degrees. These findings suggest that i) exponential kinetics may be a shared feature of most, if not all INDs, ii) the chance of rescuing a mutant neuron with treatment does not diminish with age, and iii) the kinetics are most simply accomodated by a biochemical model in which mutation imposes a mutant steady-state on the neuron, a state in which the risk is increased that homeostasis will fail, triggering apoptosis.

Program Nr: 1295 from the 1999 ASHG Annual Meeting

A new phenotypic variant of X-linked adrenoleukodystrophy (ALD) presenting in the newborn period. *D. Corzo[1], G.F. Cox[1], N.M. Hobbs[2], E.J. Quackenbush[1], P. Watkins[3], A. Moser[3], H. Moser[3]*. 1) Div Genetics, Dept Medicine, Children's Hospital, Boston, MA; 2) Div General Pediatrics, Dept Medicine, Children's Hospital, Boston, MA; 3) Kennedy-Krieger Institute, Baltimore, MD.

ALD protein (ALDP) is a peroxisomal membrane protein thought to be involved in the import or anchoring of very long chain fatty acid Co-A synthetase (VLCFAS). Its deficiency leads to impaired degradation and consequent accumulation of saturated VLCFA. Several distinct phenotypes of X-linked ALD exist with ages of onset usually after age 3. We describe an unusual case with a severe neonatal onset.

A 3.3 kg full-term male was born to non-consanguineous French Canadian parents. Hypotonia, poor suck, cholestatic jaundice, and bilateral sensorineural hearing loss were noted in the newborn period and followed by failure to thrive (FTT). Physical exam revealed jaundice, hepatomegaly, hypotonia, and no significant dysmorphism. At two months, he experienced seizure-like activity and had an abnormal EEG. He died at four months from complications of severe FTT, gastrointestinal bleeding, and respiratory failure. .

Laboratory testing revealed increased plasma VLCFA with normal pipecolic acid, phytanic acid, and RBC plasmalogens. In cultured fibroblasts, VLCFA were increased, while phytanic and pristanic acid oxidation and plasmalogen synthesis were normal. Blood and urine total bile acids were increased without detectable di- or trihydroxycholestanoid acids. Brain MRI showed diffuse increased T1 hyperintensities in white matter. Brain MR spectroscopy showed prominent signals consistent with lipid. Electron microscopy of liver revealed peroxisomes of normal size and number. Catalase distribution in fibroblasts was normal. Immunofluorescent staining in fibroblasts revealed a normal pattern for acyl CoA oxidase, absent ALDP, and an unusual pattern of staining for VLCFAS.

This case represents a novel and severe neonatal form of X-linked ALD. Identification of the specific mutation responsible for this phenotype may provide insights into ALDP function.

Program Nr: 1296 from the 1999 ASHG Annual Meeting

Structural basis of phenylketonuria. *R.H. Cotton[1], I.G. Jennings[2], B. Kobe[2].* 1) Mutation Res Ctr, Fitzroy, St Vincents Hosp, Melbourne, Australia; 2) Structural Biology Laboratory, St. Vincent's Institute of Medical Research, Fitzroy, Melbourne, Australia.

Phenylalanine hydroxylase (PAH) is the enzyme that converts phenylalanine to tyrosine as a rate limiting step in phenylalanine catabolism and protein and neurotransmitter biosynthesis. Over 300 mutations have been identified in the gene encoding PAH that result in a deficient enzyme activity and lead to the disorders hyperphenylalaninemia and phenylketonuria. The determination of the crystal structure of PAH now allows the determination of the structural basis of any mutation resulting in PAH deficiency. We present an analysis of the structural basis of 105 classified mutations and the mutations for which the activity of the corresponding expressed proteins has been tested in vitro. We find that the mutations can be classified into six categories, based on the distinct structural and functional effects of the mutations in each category. Missense mutations and small amino acid deletions account for three categories: active site mutations, dimer interface mutations, and domain structure mutations. The remaining three categories are truncation mutations (caused by the introduction of premature stop codons), frameshift mutations, and splicing mutations. The structural information will help predict the likely effects of unclassified and newly discovered mutations.

Program Nr: 1297 from the 1999 ASHG Annual Meeting

A novel major deletion in the alanine glyoxylate aminotransferase gene in type 1 hyperoxaluria. *M.B. Coulter-Mackie[1,2], G. Rumsby[3], J. Toone[1,2], D. Applegarth[1,2]*. 1) Biochemical Diseases Lab, British Columbia Children Hosp, Vancouver, BC, Canada; 2) Department of Pediatrics, University of B.C., Vancouver, Canada; 3) Department of Chemical Pathology, UCL Hospitals, London, UK.

Primary hyperoxaluria type 1 (PH1) is an autosomal recessive disease characterized by progressive kidney failure due to deposition of insoluble calcium oxalate and caused by a deficiency of the liver peroxisomal enzyme alanine glyoxylate aminotransferase (AGT). If AGT is deficient, glyoxylate is converted to insoluble calcium oxalate salts. There is a common polymorphism, the minor allele, in the AGT gene (AGXT) that occurs in up to 20% of the general population. Detection of this allele is useful for diagnostic linkage studies. The minor allele itself is without clinical consequences but when overlaid with a G170R missense alteration the AGT mislocates to the mitochondria. PH1 was confirmed in our patient at age 5 by AGT assay of a liver biopsy. AGT was about 14% of normal consistent with the presence of a mislocation mutation. DNA analysis was performed on the patient, parents and unaffected brother. Typing of the major and minor alleles using markers in intron and exon 1 suggested a deletion of at least part of AGXT. A G170R mislocation mutation in exon 4 was identified in the patient and her father but not the mother but the patient appeared to be homozygous. The results for G170R and the major/minor allele assignments were consistent with deletion and hemizygosity of the affected region of the gene. Analysis of an intron 4 VNTR was consistent with the presence of a deletion extending at least as far as intron 4. The linkage of 3 extragenic microsatellite markers, D2S140, D2S125, and D2S395 was not disrupted. The family members were informative for 2 polymorphisms in AGXT: C1342A in the 3'UTR and A776G in exon 6. Heterozygosity suggests the gene is intact from exon 6 onwards. A combination of PCR and testing for the A776 suggests the 3' end of the deletion is within exon 5 or intron 5. The 5' end is upstream from exon 1. We are using a combination of long range PCR and Southern blotting to delineate the extent of the deletion. This represents the first major deletion documented in AGXT.

Program Nr: 1298 from the 1999 ASHG Annual Meeting

X-linked ichthyosis due to partial deletions of the steroid sulfatase gene. *S. Cuevas-Covarrubias[1], M. Valdes-Flores[2], A. Jimenez-Vaca[1], S. Kofman-Alfaro[1]*. 1) Genetica, Hosp General de Mexico, Mexico, DF, Mexico; 2) Instituto Nacional de Ortopedia, Mexico, DF, Mexico.

X-linked ichthyosis (XLI) is an inherited disease due to steroid sulfatase (STS) deficiency. Onset is at birth or early after birth with dark, regular and adherent skin scales in extremities and trunk. 85-90% of XLI patients have a complete deletion of the STS gene, located on Xp22.3 However, three partial deletions have been reported in the literature, one spanning exons 2-5 and the other two lacking the 3' end of the STS gene. This study describes two partial deletions of the STS gene in two non-related XLI cases. Our initial sample included 90 subjects that were classified as XLI patients through STS assay in leukocytes using 7-3H-dehydroepiandrosterone sulfate as a substrate. Amplification of the 5' and 3' ends of the STS gene was initially performed in all subjects through PCR. Only two patients showed partial amplification of the STS gene, one observed at the 5' end and the other at the 3' end. In these cases, amplification of exons 2 and 5-9 of the STS gene was subsequently performed. The patient with the deletion of the 3' end only amplified exon 1 and the patient that lacked the 5' end only amplified exons 6-10. We informed two novel intragenic deletions of the STS gene causing XLI.

Program Nr: 1299 from the 1999 ASHG Annual Meeting

Topological analysis of the polytopic membrane glycoprotein Niemann-Pick C1. *J.P. Davies, R. Gordon, Y.A. Ioannou.* Human Genetics, Mount Sinai School of Medicine, New York.

Niemann-Pick type C1 (NPC1) is an inherited lysosomal cholesterol storage disorder which results in progressive neurodegeneration and premature death. Recently, the defective gene was identified and is predicted to be a multitopic membrane glycoprotein. NPC1 shares only limited similarity to other known proteins; most notably, a small region is homologous to the putative sterol-sensing domains (SSD) of hydroxymethylglutaryl-Coenzyme A reductase (HMGR) and sterol regulatory element binding-protein cleavage-activating protein (SCAP), two cholesterol-regulated genes. However, the precise function(s) of NPC1 are unknown. To assist our studies we have determined its topology, using glycosylation/ deglycosylation analyses, epitope tagging using the Flag octapeptide sequence, and immunoelectron-microscopy. These studies revealed that NPC1 contains 13 transmembrane domains; 3 large lumenal hydrophilic regions; a single large cytoplasmic loop; a lumenal amino terminus and a cytoplasmic carboxy-terminal tail. Interestingly, the putative SSD of NPC1 is oriented exactly as in HMGR and SCAP, whose topologies have been determined. This strongly suggests that both the protein sequence and its orientation are functionally important in all three proteins. These data will significantly aid our future studies to determine the exact function(s) of this large membrane glycoprotein.

Program Nr: 1300 from the 1999 ASHG Annual Meeting

Mutation analysis of the mevalonate kinase gene in patients with Hyper-IgD and periodic fever syndrome (HIDS): Identification of 3 new mutations. *M. Delpech[1], L. Cuisset[1], JPH. Drenth[1,2], SD. van der Velde Visser[3], G. Grateau,[1,4], JWM. van der Meer[2]*. 1) Genetique Moleculaire Humaine, EA2500, Inst. Cochin Genet Moleculaire, Paris, France; 2) Department of Medicine, Division of General Internal Medicine, University Hospital St. Radboud, Nijmegen, The Netherlands,; 3) Department of Human Genetics, University Hospital St. Radboud, Nijmegen, The Netherlands,; 4) Service de Medecine Interne, L'Hotel-Dieu (AP-HP), Paris, France.

The hyperimmunoglobulinemia D and periodic fever syndrome (HIDS) is a rare familial autosomal recessive disorder featuring recurrent fever. Typical attacks occur every 4 to 8 weeks, and symptoms include lymphadenopathy, abdominal distress, joint involvement and skin lesions. They are associated with an intense acute phase response and all HIDS patients have constantly high serum IgD values (> 100 U/ml) with attacks but also during intervals. We and others recently established mevalonate kinase (ATP:mevalonate 5-phosphotransferase, EC 2.7.I.36) (MVK) as the causative HIDS gene. MVK follows 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase in the synthetic pathway of cholesterol. Four missense mutation (H20P, P167L, I268T, V377I), a 92 bp loss stemming from a deletion or from exon skipping, and the absence of expression of one allele were found in a total of fourteen patients. The V377I (G->A) mutation appeared to be the most common mutation in HIDS. Here we present a study on the mutation spectrum of the MVK gene on nine additional patients. Mutations were found in 18 alleles (100%), of which 8 (44%) carried the V377I (G->A) mutation and 4 (22%) carried the I268T (T->C) mutation. In the six remaining alleles, five different mutations were found. Only two of them, (H20P and the absence of expression of one allele) have been described before. All the three new mutations represent missense mutations and are H20N, R215Q and G326R. These result confirm that the V377I (G->A) mutation is the most common mutation in HIDS and reveal that the I268T (T->C) mutation is also frequent.

Program Nr: 1301 from the 1999 ASHG Annual Meeting

Investigation of functional domains in human lysosomal b**-hexosaminidase A.** *E. Denis[1,2], F. Kaplan[1,3,4], B. Boulay[1], P. Hechtman[1,2,3,4].* 1) McGill University-Montreal Children's Hospital Research Institute; 2) Department of Biology, McGill University; 3) Department of Human Genetics, McGill University; 4) Department of Pediatrics, McGill University, Montreal, Quebec, Canada.

The major b-hexosaminidase isozymes in humans are Hex A (deficient in Tay-Sachs disease, TSD), an ab heterodimer and Hex B (deficient in Sandhoff disease) a bb homodimer. Hex S, the aa homodimer is physiologically unstable. Mature a and b subunits share 60% sequence identity. The b subunit hydrolyzes neutral substrates. The a subunit hydrolyzes neutral (4MUG) and charged substrates (4MUGS). Only Hex A hydrolyzes the natural substrate, G_{M2} ganglioside, in the presence of the G_{M2} activator protein (AP). We investigated regions of the a and b subunits involved in AP binding, subunit dimerization, and substrate specificity. We constructed chimeric cDNAs: $a_{\mathbf{1/2}}b_{\mathbf{1/2}}$ comprises the 5' 50% of the a-cDNA and the corresponding 3' 50% of the b-cDNA; $b_{\mathbf{3/4}}a_{\mathbf{1/4}}$ comprises the 5' 75% of the b-cDNA and the 3' 25% of the a-cDNA. Chimeric cDNAs were expressed in a TSD neuroglial cell line which produces no endogenous a subunits. The chimeric isozymes were chromatofocused and assayed for hydrolysis of a) 4MUG , b) 4MUGS and c) G_{M2} ganglioside (in the presence of AP/detergent). Transfection of both cDNA constructs lead to expression of homodimeric and heterodimeric chimeric proteins, albeit at lower yields than transfection of normal a-cDNA. $(b_{3/4}a_{1/4})_2$ (homodimer) and $(b_{3/4}a_{1/4})b$ (heterodimer) hydrolyzed 4MUG but neither hydrolyzed 4MUGS or G_{M2} ganglioside. By contrast, $(a_{1/2}b_{1/2})_2$ and $(a_{1/2}b_{1/2})b$ hydrolyzed both 4MUG and 4MUGS. However, while G_{M2} ganglioside hydrolysis by these isozymes could be detected in the presence of detergent (which can replace AP in vitro) no activity was observed in the presence of the AP. These results suggest that a) both constructs contain sufficient information to form both heterodimeric and homodimeric chimeric proteins b) $b_{3/4}a_{1/4}$ is absent in an a domain essential to the hydrolysis of negatively charged substrates and c) $a_{1/2}b_{1/2}$ is absent in an a domain essential to AP binding. Future studies will allow us to more precisely define the a subunit regions essential to these functions.

Program Nr: 1302 from the 1999 ASHG Annual Meeting

Obesity, Diabetic Ketoacidosis (DKA), Malignant Hyperthermia (MH), and Rhabdomyolysis (RM): A New Phenotype of Carnitine Palmitoyl Transferase Type II (CPTII) Deficiency? *J. DiPaola, P.H. Schwartz, N. Anas, T.J. Butler, J. Cappon, S.A. Stein.* Crit Care, Neurol and Brain and Tissue Bank, Child Hosp Orange County , Orange, CA.

Two, unrelated, obese, 15-year-old hispanic boys presented with a clinical picture of new onset DKA after a 1 - 2 week history of fatigue, malaise, polydipsia and polyuria. The clinical course of both patients was catastrophic and led to cardiac arrest. One (A) was successfully resuscitated. The other (B) died in multisystem organ failure. The laboratory values for both were also very similar. Glucose levels at presentation were 1400 mg/dL (A) and 1900 mg/dL (B). Severe metabolic acidosis was noted. Creatine phosphokinase levels reached 47,000 U/L (A) and 68,000 U/L (B). The Mb isoenzyme was elevated but the enzyme/isoenzyme ratios were normal. Both patients had abnormal EKGs, most likely related to electrolyte disturbances. They required endotracheal intubation and high doses of inotropes for cardiovascular support. Both patients had myoglobinuria and developed severe renal failure that required hemodialysis. They also developed MH, measured rectally at 42.8 C (A) and 42.6 C (B), successfully treated with dantrolene. Muscle biopsy of A showed non-specific diffuse myofiber degeneration and regeneration typical of RM; enzymatic testing showed 10% residual CPTII activity which was negative for the S113L mutation. Autopsy of B revealed multisystem organ failure, severe hemorrhagic pancreatitis, and fatty metamorphosis of the liver with hepatomegaly and cirrhosis. The association of DKA and RM, particularly in adults, is well known. There have also been reports of MH in association with diabetes. While RM has been reported in CPTII deficiency, it is usually associated with strenuous exercise or starvation. To our knowledge there has been only one other recognized case similar to those presented here. These cases raise the possibility that CPTII deficiency is the underlying cause for the entire clinical picture and that appropriate preventive measures to avoid MH and a shift to fat metabolism should be taken in addition to measures to control DKA.

Program Nr: 1303 from the 1999 ASHG Annual Meeting

Alterations in SREBP2 expression in Niemann Pick C (Npc1$^{-/-}$) LDL receptor knockout mice. *R.P. Erickson, W.S. Garver, M. Midura-Kiela, R.A. Heidenreich.* University of Arizona College of Medicine, Angel Charity for Children - Wings for Genetic Research, Steele Memorial Children's Research Center, Department of Pediatrics, Section of Medical and Molecular Genetics, Tucson, AZ.

Niemann-Pick disease type C (NPC) is a devastating inborn error of metabolism with hepatic and neurological symptoms. Early biochemical and pathological analyses of both mouse and human NPC tissues and cell lines reveal a massive accumulation of unesterified cholesterol, the source of which is low density lipoprotein (LDL). The sterol regulatory element binding proteins (SREBPs) are membrane-bound proteins found in the endoplasmic reticulum (ER) membrane that regulate cholesterol synthesis, including LDL receptor expression. Using mouse liver homogenates, we provide evidence indicating an upregulation of SREBP2 in the NPC1$^{-/-}$/LDLR$^{-/-}$ mouse compared to NPC1$^{-/-}$/LDLR$^{+/+}$ mice of the same age. However, while the amount of SREBP2 is downregulated in NPC1$^{-/-}$/LDLR$^{+/+}$ mice with increasing age, the amount of SREBP2 is upregulated in NPC$^{-/-}$/LDLR$^{+/+}$ mice with increasing age. These results suggest a functional relationship between NPC1$^{-/-}$/LDLR$^{+/+}$ and SREBP2 in the maintenance of intracellular cholesterol levels.

Program Nr: 1304 from the 1999 ASHG Annual Meeting

Prenatal diagnosis of repiratory chain disorders. *L. Faivre, D. Chretien, J.C. von Kleist-Retzow, V. Cormier-Daire, A. Rötig, P. Rustin, A. Munnich.* Department of Genetics, Hopital Necker Enfants Malade, Paris, France.

A reliable prenatal diagnosis is an important goal for genetic counseling in mitochondrial respiratory chain deficiency. In the absence of detectable mitochondrial DNA (mtDNA) mutations, measurement of respiratory chain enzymes activities represents the only hope of prenatal diagnosis, especially as only a few nuclear disease genes have been identified. We carried out enzymatic prenatal diagnosis in 16 pregnancies from 9 unrelated couples using uncultured choriocytes and/or amniocytes. Inclusion criteria were i) a previous child with a fully documented disease, ii) absence of mtDNA mutation, and iii) the existence of an enzyme defect in cultured skin fibroblasts of the proband. In 5/16 pregnancies, the fetus was found affected, the pregnancy was terminated and the diagnosis was further confirmed on postabortion tissues. In 9/16 cases, the tests were normal, the pregnancies were continued and resulted in the birth of an unaffected healthy child. Unfortunately, in 2/16 cases, a fetus which appeared normal from early prenatal diagnosis, turned out to be affected with the disease. From this experience, we conclude that an abnormal result should be considered reliable, but a normal result between 8 and 12 weeks of gestation does not give a definitive clue as to the child condition after birth. We therefore suggest the following guidelines: i) a choriocentesis or an amniocentesis should be proposed in early pregnancy, ii) a second amniocentesis at 28 weeks of gestation should be offered in order to avoid false negative result because of late expression of the disease, iii) a careful and repeated ultrasound survey in the third trimester, and iv) prenatal diagnosis should not be proposed for a complex I deficiency as this enzyme activity cannot be accurately measured in fetal cells.

Program Nr: 1305 from the 1999 ASHG Annual Meeting

Analysis of the flavin-containing monooxygenase (*FMO3*) gene for mutations in Australia identifies a high carrier frequency for TMAuria and a novel deletion in a Greek patient. *S.M. Forrest[1], M.A. Knight[1], B.R. Akerman[2], E.P. Treacy[2]*. 1) Murdoch Institute, Royal Children's Hospital, Parkville, Victoria, Australia; 2) C.R. Scriver Biochemical Genetics Unit, Montreal Children's Hospital, Quebec, Canada.

Trimethylaminuria (TMAuria) is an inborn error of metabolism resulting from the inability to metabolize the dietary-derived odorous amine trimethylamine by N-oxygenation. Affected individuals consequently suffer a severe body odor with associated psychosocial conditions. This inborn error of metabolism is uncommon and spread non-randomly throughout specific populations. The majority of affected Australian individuals are of British or Irish origin. We have recently shown that mutations of the flavin-containing monooxygenase type 3 gene, *FMO3* - the catalytically dominant active liver isoform, cause this condition. Two common mutations were identified in our Australian cohort, E305X and P153L, accounting for 88% of mutations in Australians of British/Irish origin. Two other mutations, M66I and R492W, were found in a compound heterozygous state in one other Australian patient. Screening for the alleles E305X and P153L in 57 unaffected controls has indicated that 7 individuals carry P153L giving a carrier frequency of 1/8 and suggesting that this condition has a significantly higher incidence in Australia.

In a Greek individual with severe TMAuria we have now identified a novel homozygous deletion of exons 1 and 2 of the *FMO3* gene encompassing 12kb of genomic DNA. This deletion was not detected in 40 Greek control chromosomes. As this deletion should result in complete absence of functional FMO3, this patient will be unable to metabolise a number of other FMO3 substrates in addition to TMA. Thus, in addition to the common *FMO3* gene mutations causing TMAuria in Australians of British/ Irish origin, other 'private mutations' may be segregating in Australians of diverse ethnic backgrounds.

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Program Nr: 1306 from the 1999 ASHG Annual Meeting

Molecular study of Persistent Hyperinsulinemic Hypoglycemia of Infancy (PHHI): genetic heterogeneity and phenotype-genotype correlations in a series of 143 patients. *J.C. Fournet[1,2], C. Mayaud[1], M.S. Gross-Morand[1], P. de Lonlay[1,3], V. Verkarre[1,2], J. Rahier[5], F. Brunelle[4], J.J. Robert[3], C. Nihoul-Fekete[5], J.M. Saudubray[3], C. Junien[1]*. 1) INSERM UR 383, Hosp Necker-Enfants Malades, Paris, France; 2) Department of Pathology,Hosp Necker-Enfants Malades, Paris, France; 3) Pediatrics-Metabolism, Hosp Necker-Enfants Malades, Paris, France; 4) Pediatric surgery, Hosp Necker-Enfants Malades, Paris, France; 5) Department of Pathology, Brussels, Belgium.

Persistent Hyperinsulinemic Hypoglycemia of Infancy (PHHI) ("nesidioblastosis") is characterized by profound hypoglycemia due to inappropriate hypersecretion of insulin and is linked to, at least, two morphological forms: a focal form and a diffuse one. Focal forms are characterized by a somatic loss of the maternal chromosomal region 11p15 carrying imprinted genes and the genes SUR1 and KIR6.2 coding for the two subunits of the K+ATP channel of B cell in FoPHHI but not in DiPHHI. In order to determine the extent of genetic heterogeneity and to look for potential phenotype/genotype correlation, we have screened 143 patients with PHHI for mutations in 27 exons on 39 of the SUR1 gene and of the entire KIR6.2 gene. In 10 focal forms out of 27 (37%), we have found a paternally transmitted mutation of the gene SUR1. In one patient, we have found the first KIR6.2 mutation associated with a focal form. This paternal mutation is reduced to homozygosity by loss of the maternal allele, restricted to the lesion. In 116 diffuse forms and in non operated cases, we have found 26 SUR1 mutations (23 undescribed) (22,4%) and 2 new KIR6.2 mutations (1,7%). Furthermore we have developed SUR1 and KIR6.2 mutation databases (hhtp://www.umd.necker.fr:2007) which allowed us to define mutation-rich regions and phenotype-genotype correlations.

Program Nr: 1307 from the 1999 ASHG Annual Meeting

DNA and enzyme diagnostics for tripeptidyl peptidase I (CLN2) involved in classical late infantile neuronal ceroid lipofuscinosis. *P.F. Franken[1], J.L.M. Keulemans[2], M.H. Breuning[1], O.P. Van Diggelen[2], P.E.M. Taschner[1]*. 1) Department of Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands; 2) Department of Clinical Genetics, Erasmus University Rotterdam, Rotterdam,The Netherlands.

The neuronal ceroid lipofuscinoses (NCL) are a heterogeneous group of recessive neurodegenerative disorders with an estimated incidence of 1: 50,000 in the Netherlands. These disorders are characterized by visual failure, seizures, mental deterioration, and lysosomal storage of autofluorescent lipopigments. The differential diagnosis of NCL is difficult, because many different forms exist. Recently, 4 genes involved in NCL have been identified, including two genes for the genetically heterogeneous late infantile NCL. The gene involved in classical late infantile NCL (LINCL) or Jansky-Bielschowsky disease (MIM 204500), CLN2, encodes the lysosomal enzyme tripeptidyl peptidase I (previously known as pepstatin-insensitive proteinase). Routine diagnostic procedures for lysosomal storage disorders should commence with enzyme analysis, which will detect enzyme defects caused by known as well as unknown mutations in the corresponding genes. Recently, a new fluorogenic enzyme assay with increased sensitivity has been developed for tripeptidyl peptidase I. The assay is three orders of magnitude more sensitive than the original assay of Sleat et al. (Science 277:1802 (1997)) and has been validated on classical LINCL patients with known CLN2 mutations. The new assay has been used to confirm the clinical diagnosis of 7 LINCL patients. Subsequently, the screening for mutations in the CLN2 gene can be limited to LINCL patients who are tripeptidyl peptidase I deficient. We have used single strand conformation analysis of genomic PCR products containing the different CLN2 exons, followed by direct sequencing, to identify mutations in our collection of 26 independent LINCL patients. Prior to the new enzyme assay, six patients were found to carry the IVS5-1 G®C splice site mutation on 10 chromosomes.

Program Nr: 1308 from the 1999 ASHG Annual Meeting

Aortic root calcification precedes obstructive coronary artery disease in children with homozygous familial hypercholesterolemia. *C.A. Friedrich[1], D.M. Kolansky[2], S.M. Paridon[3], B.J. Clark[3], E.S.C. Brown-Parr[1], J.M. Wilson[1], D.J. Rader[2].* 1) Medicine/Medical Genetics, University of Pennsylvania, Philadelphia, PA; 2) Medicine/Cardiology; 3) Pediatrics/Cardiology.

Homozygous familial hypercholesterolemia (HFH) is a rare disorder due to LDL-R gene mutations associated with markedly accelerated atherosclerosis. Most patients develop coronary artery disease (CAD) by their teens or twenties but frequently experience a myocardial infarction or sudden death without preceding symptoms. There are no clear guidelines for the cardiovascular evaluation of asymptomatic patients with HFH. Electron beam CT scanning (EBCT) for coronary artery calcification is a non-invasive method for quantitating coronary plaque burden and may predict the risk of CAD in adults. We therefore sequentially evaluated 22 children and adolescents with HFH with EBCT scans, and with coronary artery angiograms. This is the largest FH cohort studied with EBCT. We defined CAD as any lesion that caused more than 50% stenosis of a coronary artery. Ages at initial evaluation ranged from five to 17 years old. Five patients had at least one coronary artery stenosis greater than 50% at the time of their initial EBCT. Only one of these five had coronary artery calcification. However, four had substantial calcification present in the ascending aorta and aortic arch, and the fifth patient developed aortic calcification one year after his first evaluation. Of those patients without significant coronary artery lesions, fourteen had calcification in the ascending or thoracic aorta. Two of these 14 patients also had coronary artery calcification. Three children (ages 5 to 14) had no aortic or coronary artery calcification, and no CAD. Three of the 14 originally found to have calcification without significant CAD developed CAD stenoses greater than 50% over the next three years. We conclude that aortic root calcification usually precedes development of clinically significant coronary artery atherosclerosis in children with HFH. EBCT may therefore be a useful non-invasive tool in evaluating asymptomatic HFH patients. Asymptomatic patients without aortic root calcification are unlikely to have CAD on angiography.

Program Nr: 1309 from the 1999 ASHG Annual Meeting

Mammalian *PEX13*: cDNA cloning by functional complementation on newly identified peroxisome assembly-defective Chinese hamster ovary cell mutants, characterization, and mutation analysis. *Y. Fujiki[1,2], R. Toyama[1], S. Mukai[1,2], A. Itagaki[1], N. Shimozawa[3], R.J.A. Wanders[4], S. Tamura[1].* 1) Department of Biology, Graduate school of Science, Kyushu University, Fukuoka 812-8581, Japan; 2) CREST, Japan Science and Technology Corporation, Tokyo, 170-0013, Japan; 3) Department of Pediatrics, Gifu University Faculty of Medicine, Gifu 500-8076, Japan; 4) Department of Clinical Biochemistry and Pediatrics, Academic Medical Centre, University of Amsterdam, P.O. Box 22700, 1100DE, Amsterdam, The Netherlands.

We isolated peroxisome biogenesis mutants, ZP128 and ZP150, from rat *PEX2*-transformed Chinese hamster ovary cells, using the 9-(1'-pyrene)nonanol/ultraviolet (P9OH/UV) method. The mutants lacked morphologically recognizable peroxisomes and showed a typical peroxisome assembly-defective phenotype such as severe loss of catalase latency and high sensitivity to 12-(1'-pyrene)dodecanoic acid/UV treatment. ZP128 and ZP150 were found to belong to a recently identified complementation group H, by complementation group analysis by means of *PEX* cDNA transfection as well as cell fusion. Human cDNA encoding peroxin Pex13p was isolated from a liver cDNA library by functional complementation of peroxisome deficiency in ZP128, using a transient transfection assay. Pex13p comprising of 403 amino acids is a peroxisomal integral membrane protein, exposing both the N-terminal part and C-terminal src homology 3 (SH3)-domain to the cytosol. A stable transformant of ZP128 with the *PEX13* was morphologically and biochemically restored for peroxisome biogenesis. Chinese hamster *PEX13* was also isolated, of which deduced amino acid sequence showed 93%, 26%, and 25% identity with human, *Saccharomyces cerevisiae*, and *Pichia pastoris* Pex13p, respectively. Therefore, ZP128 and ZP150 are the first mammalian cell lines with a phenotype of impaired *PEX13*. Analysis of mutation(s) in *PEX13* of mutant ZP150 is underway.

Program Nr: 1310 from the 1999 ASHG Annual Meeting

Elevated 1-methyl histidine in a subgroup of autism patients. *D.K. Gavrilov, N. Takahashi, M. Muskopf, C. Wang, J.H. Miles, R.E. Hillman.* Depts of Biochemistry, Child Health and Neurology, Univ of Missouri, Columbia, MO.

Autism is highly genetic neuropsychiatric disorder with poorly understood biologic basis. Of 182 consecutive autism probands screened for the presence of 1-methyl histidine (1-MeHis) in the urine by qualitative 2-dimensional electrophoresis, 16.5% (30/182) had elevated levels. Quantitative 1- MeHis levels were obtained by HPLC. The high 1-MeHis group was compared with 2 control groups: 21 consecutive autism probands without elevated 1-MeHis and 44 consecutive non-autism patients referred for metabolic screening. The levels of 1-MeHis were normalized to the urine creatinine. The non-autism control group mean ± 2SD was used to establish the normal range (13 ± 26 mmol/ mmol creatinine). The 1-MeHis distribution in the autism control group was not significantly different (16 ± 20). The 1-MeHis mean in the elevated 1-MeHis autism probands was statistically higher than in either control group (t-test values of 6.795, $p<0.001$ and 5.55, $p<0.001$ respectively). **Results:** A group of autistic patients was identified with elevated excretion of 1-MeHis and a correlation with the 1 MeHis levels was noted. This subgroup differed significantly from the remainder of autism probands in four areas: 1) the male:female ratio was higher with highest values in a subgroup with 1-MeHis>100 (16:1 vs 7.5:1); 2) the incidence of seizures is higher (12/28 vs. 2/17, $p<0.05$), (8/17 in the subgroup with 1-MeHis>100; 3) the family history of attention deficit hyperactivity disorder (ADHD) was lower than in the control autistic group ($p<0.05$) (0/17 in the subgroup with 1-MeHis>100 vs 6/20 or 30% in the control subgroup and 4) significantly higher incidence of dismorphic clinical features - 52.9% vs 28.6% in the control group. A tendency towards IQ<70 was noted in the subgroup with 1-MeHis > 100 (13/14 or 92.9% vs 12/17 or 70.6%). Follow up of two patients with extremely high levels of 1-MeHis have shown at least 10 times reduction of 1-MeHis excretion after treatment with combination of vit. B_6, folic acid and dimethyl glycine. Delineation of homogeneous groups is necessary for identification of disease causing genes.

Program Nr: 1311 from the 1999 ASHG Annual Meeting

Increased thickness of large arteries: a new marker of Fabry's disease. *D.P. Germain[1], O. Lidove[2], J.P. Grunfeld[2], X. Jeunemaitre[1], X. Girerd[3], V. Remones[1], S. Laurent[3], P. Boutouyrie[3].* 1) Genetique, Hopital Broussais, Paris, France; 2) Nephrologie, Hopital Necker, Paris, France; 3) INSERM U337, Hopital Broussais, Paris, France.

Fabry's disease is an X-linked inborn error of sphingolipid metabolism due to defects in the lysosomal alpha-galactosidase A gene. The enzymatic defect results in systemic deposition of uncleaved glycosphingolipids in the lysosomes of vascular endothelial and smooth muscle cells in most tissues. The major clinical manifestations in FD are primarily due to a progressive small vessels pathology with angiokeratoma, renal failure and vasculopathy of the heart and brain. Death usually occurs in adult life from renal, cardiac or cerebral complications of vascular disease. Early indicators measuring the progressive accumulation of glycosphingolipids could be of high interest for the care of FD patients. We hypothesized that this could be possible at the site of large arteries by using recently developed high precision echotracking devices. Nineteen FD male patients, without end-stage renal disease, were compared with 22 male controls (Co) matched for age, renal function and blood pressure (BP). Common carotid (CCA) and radial artery (RA) diameter (Di), intima-media thickness (IMT), and distensibility (Dist) were determined with high definition echotracking system and aplanation tonometry. An increased radial artery thickness was associated to Fabry's disease ($p<0.001$) independently of age and mean BP. RA Dist was increased two-fold in FD patients (9.1±0.9 vs 4.8±0.7 $kPa^{-1}.10^3$). RA IMT increased with age in FD patients and controls, but the slope was three-fold higher in FD patients (6.2±2.0 vs 1.8±0.6 mm/year). CCA IMT was significantly increased in patients with FD ($p<0.001$), without significant difference in diameter and distensibility. In conclusion, FD patients have an accelerated thickening of large arteries, which is more prominent in muscular (RA) than elastic (CCA) arteries. This new noninvasive hallmark could be of particular interest for the monitoring of future enzyme replacement therapy.

Program Nr: 1312 from the 1999 ASHG Annual Meeting

Respiratory activity, antioxidant defences, cell growth and apoptosis in human ubiquinone-deficient skin fibroblasts. *V. Geromel, N. Khadom, D. Chretien, A. Munnich, A. Rotig, P. Rustin.* INSERM U393, Hopital Necker, Paris, France.

Beside its well established role in electron transfer in the mitochondrial respiratory chain, ubiquinone has been claimed to exert, through its antioxidant properties, a major role in controlling free radical level in the cell, in the apoptotic process, possibly acting as a control partner in aging. We previously reported a widespread ubiquinone deficiency in two siblings presenting as encephalomyopathy. No ubiquinone could be detected in the cultured skin fibroblasts that accumulated farnesyl-pyrophosphate, an intermediate compound in ubiquinone synthesis. We next investigated the consequence of the ubiquinone deficiency on cell respiration and oxidation of various substrates in detergent-permeabilized fibroblasts and found a surprisingly high level of residual activities, suggesting that the pool function of ubiquinone may be necessary only under specific conditions. Comparing cell growth between ubiquinone-deficient and control fibroblasts revealed a limited decrease of cell growth in patient fibroblasts. Accordingly, DNA fragmentation tested with the TUNEL reaction and nuclear morphology as revealed by Hoechst 33242 did not significantly differ between patient and controls. Even serum withdrawal in the culture medium did not specifically affect ubiquinone-deficient fibroblasts. Finally, antioxidant enzymes (superoxide dismutases, catalase, glutathione reductase, transferase or peroxidase) were not increased in response to the ubiquinone deficiency However, the addition of idebenone, a short chain homologue of ubiquinone prone to auto-oxidation, induced a higher lethality in ubiquinone-deficient fibroblasts, suggesting that ubiquinone may act as an antioxidant in situ in these cells. It therefore appears that in human skin fibroblasts, ubiquinone i) can act as an electron carrier in the respiratory chain independently of its pool function; ii) does not directly control cell growth through a putative regulation of the apoptotic process; iii) but may participate to the defenses of the cells against oxidative stresses.

Program Nr: 1313 from the 1999 ASHG Annual Meeting

Characterization of *hSCO2* mutations involved in cytochrome oxidase deficiency. *D.Moira Glerum[1], L. Papadopoulou[2], E. Schon[2], D. Adams[1]*. 1) Dept of Medical Genetics, University of Alberta, Edmonton, Alberta, Canada; 2) Dept of Neurology, Columbia University, New York, NY 10032.

Defects in mitochondrial function are increasingly being identified as the cause underlying a number of different neurodegenerative disorders. A subset of these disorders arise due to specific deficiencies in cytochrome oxidase (COX). COX is the terminal electron acceptor in the mitochondrial respiratory chain and consists of 13 polypeptide subunits, along with 2 heme molecules and 3 copper atoms. Provision of copper to the apoenzyme is a prerequisite for assembly of a functional enzyme. Studies in the yeast *Saccharomyces cerevisiae* have shown that some 40 nuclear gene products are required. In particular, Cox17p and Sco1p are involved in providing copper to mitochondria and COX (Glerum et al, 1996). The human homologs for these two proteins have been identified, and recently, mutations in *hSCO2* (a human homolog of yeast *SCO1*) have been shown to segregate with COX deficiency in patients with fatal infantile cardioencephalomyopathy (Papadopoulou et al, submitted). We now demonstrate that, although highly similar, *hSCO2* is not an ortholog of yeast *SCO1*. In an effort to characterize the effects of the mutations, we have introduced the parallel mutations into the yeast *SCO1* gene and transformed the mutated constructs into a yeast *SCO1* knock-out strain. We will present data delineating the impact of these mutations on cytochrome oxidase assembly, using spectral analysis of mitochondrial hemes, COX activity assays and determination of growth rates in non-fermentable carbon sources. The results demonstrate that the mutations adversely affect COX assembly and provide an explanation for the COX deficiency seen in the patients. The creation of site-directed mutants in the yeast homologs of human genes provides an *in vivo* approach to determining the nature of mutations associated with human COX deficiencies.

Program Nr: 1314 from the 1999 ASHG Annual Meeting

Human Congenital Hypotransferrinemia: case report and molecular studies. *S. Goldwurm[1], C. Casati[1], S. Strada[1], P. Santambrogio[2], N. Venturi[1], P. Arosio[2], M. Cazzola[3], A. Piperno[1], G. Masera[1], A. Biondi[1]*. 1) Pediatric Clinic, University of MIlano-Bicocca, Monza (MI) Italy; 2) DIBIT, Scientific Institute San Raffaele, Milan, Italy; 3) Department of Internal Medicine, University of Pavia, Italy.

Human congenital hypotransferrinemia is a rare disorder characterised by the virtual absence of transferrin (Tf) in the serum. No information on the molecular basis of the defect is known. Following the identification of a child affected by hypotransferrinemia, we investigated the molecular basis of the defect and planned to characterise the DNA mutation that underlies this disease. After the diagnosis, the patient received monthly RBCs transfusions. Subsequently he was treated with plasma transfusions: the first plasma-apheresis raised the serum Tf concentration to 120 mg/dl followed by a good erythropoietic response and a normal kinetic of Tf. At present, the patient is regularly infused with plasma to keep his Hb over 10 mg/dl. He has a normal growth and no other symptoms or signs were observed. To prove that the molecular defect causing hypotransferrinemia was due to a mutation in the Tf gene, we perform an haplotype analysis of the Tf gene on 3q21-3 in the porband's family (26 individuals). We could observe the association between one affected haplotype shared with the proband and intermediate serum levels of Tf, thus indicating that the disease is inherited as a recessive trait and that the mutation is in the Tf gene. A Southern analysis excluded the presence of gross deletions or rearrengements, while Northern analyses on PBL did not detect a Tf mRNA in controls as well as in the patient. The amount of serum Tf in our patient was confirmed to be around 3 mg/dl by an ELISA assay, approximately 1/100 of normal values. In a Western analysis most of the patient's Tf run at the normal level, but a smaller band was detected. These data suggest that two different mutations are present in the 2 alleles, one in the regulatory region of the gene reducing the expression of an otherwise normal protein, the other in the coding region affecting the structure and therefore the processing of the protein.

Program Nr: 1315 from the 1999 ASHG Annual Meeting

Hetereogeneity of the MELAS3243 mutation. *O. Guardamagna[1], A. Peduto[1], C. Bondone[1], S. Baglieri[1], M. Spada[1], E. Lamantea[2], F. Carrara[2], M. Zeviani[2].* 1) Pediatrics, Univ. di Torino, Torino, Italy; 2) Istituto Neurologico Besta, Milano, Italia.

MELAS is a progressive neurodegenerative disease whose presentation is typically characterized by mitochondrial encephalomiopathy, lactic acidosis and stroke-like episodes. We present here a 3 yrs old female patient whose history is characterized by mild psycomotor retardation, growth failure and hypotonia. She was referred to our department because affected by food refusal and worsening hypotonia, as a consequence of an intercurrent illness. She was treated with total parenteral nutrition and recovered her normal clinical condition. The neurologic examination was normal. While hospitalized she acutely started vomiting and developed a serious metabolic acidosis. Blood lactate was elevated. Clinical and radiological analysis demonstrated a picture of ileum paralitycum. This picture was later aggravated by seizure and coma till fatal outcome. The muscolar biopsy revealed the presence of "ragged red fibers", an histologic feature suggesting a mitochondrial disorder. This suspect was then confirmed by the demonstration of a defect of the respiratory chain Complex I and Complex IV (3.3 nmol/min mg, nv 15-31 and 25 nmol/min mg, nv 80-180 respectively). The mitochondrial DNA analysis allowed to identify the heteroplasmic point mutation at the nucleotide 3243, an A to G transition at the tRNALeu(UUR) gene. This particular presentation, as well as previous demonstration of this mutation in patient without neurologic disease, confirms the clinical hetereogeneity of the A3243G mutation and suggest a deficiency in the mitochondrial respiratory chain of smooth muscle cells in the present patient.

Program Nr: 1316 from the 1999 ASHG Annual Meeting

Association of a Glucocorticoid Receptor Gene Marker With Human Essential Hypertension. *V. Guerrini, S. Rutherford, D.R Nyholt, L.R Griffiths.* Genomics Res Ctr, Sch Hlth Sci, Griffith University-Gold Coast, Southport QLD, Australia.

Recently, a bi-allelic polymorphism in the glucocorticoid receptor gene (GRL) has been shown to be associated with individuals at high risk of developing hypertension and accumulation of abdominal visceral fat, a known risk factor for cardiovascular disease. The evaluate the role of GRL in essential hypertension and obesity, case-control studies were conducted using 88 hypertensive, 123 normotensive, 150 lean and 94 obese subjects. Genotypes for a highly polymorphic microsatellite marker (D5S207) located within 200 kb of the glucocorticoid receptor gene, were determined by PCR. Allele frequencies between hypertensive and normotensive groups were significantly ($P = 0.0005$) different whereas no significant differences were observed between lean and obese populations. In conclusion, the results suggest that the glucocorticoid receptor gene or perhaps another gene located in close proximity and in linkage disequilibrium with D5S207, is involved in hypertension development.

Program Nr: 1317 from the 1999 ASHG Annual Meeting

Very long chain acyl-CoA dehydrogenase deficiency: clinical phenotypes, prenatal diagnosis and four novel mutations. *G. He, B.Z. Yang, J.H. Ding, D.S. Roe, C.R. Roe.* Courtwright/Summers Inst Metab, Baylor Univ Medical Ctr, Dallas, TX.

Very long chain acyl-CoA dehydrogenase (VLCAD) deficiency, an autosomal recessive disorder, is a severe defect of mitochondrial fatty acid oxidation that presents as two clinical phenotypes: a neonatal cardiomyopathy and a mild hypoglycemia form. The proband was a two-day-old girl with sudden death. In the subsequent pregnancy, prenatal diagnosis was performed using in vitro analysis of the mitochondrial beta-oxidation pathway in amniocytes. The prenatal screening revealed C12, C14:1 and C16 accumulation indicating VLCAD deficiency. Enzyme assay confirmed the diagnosis. To investigate the mutation(s), VLCAD cDNA and genomic DNA from the cultured fibroblasts were amplified and sequenced. Two novel mutations were identified: a G1613C (Arg538Pro) and a one base deletion (A770del) resulting in a frameshift and premature termination. In another case, the plasma acylcarnitine profile of a 3 1/2 year old patient with recurrent hypoglycemia revealed an elevation of C14:1, characteristic of VLCAD deficiency. In vitro analysis and enzyme assay of the patient's fibroblasts confirmed the deficiency. The sequence analysis of the VLCAD cDNA and genomic DNA from this patient identified two novel mutations: a C953T (Pro318Leu) mutation in exon 10 and a C1194A (Tyr398Stop) mutation which created a premature stop codon in exon 12. The correlation of the mutant genotype to clinical phenotype is briefly discussed.

Program Nr: 1318 from the 1999 ASHG Annual Meeting

Revealing the molecular basis of cystinosis in Dutch patients by mutation detection in the CTNS gene. *S.G. Heil[1], N.M.J. Van der Put[1], H.G. Brunner[2], J.M.F. Trijbels[1], L.A.H. Monnens[1], H.J. Blom[1]*. 1) Pediatrics, University Hospital Nijmegen, Nijmegen, The Netherlands; 2) Human Genetics, University Hospital Nijmegen, Nijmegen, The Netherlands.

Nephropathic cystinosis is an inborn error of metabolism with an autosomal recessive inheritance pattern. Cystinosis is characterized by lysosomal storage of the amino acid cystine due to an impaired transport of cystine out of the lysosomes. Clinical features are renal Fanconi syndrome, failure to thrive and crystals in the eye-lens.

Recently, the gene responsible for membrane transport of cystine (CTNS), has been cloned. This gene encodes an integral membrane protein, which is called cystinosin. The CTNS gene has 12 exons and its 2.6 kb mRNA codes for a 367-amino acid cystine transporter with 7 transmembrane domains.

Mutation detection of European patients revealed a common 65-kb deletion, and several other mutations spread throughout the entire coding region.

In the present study, we observed the common 65-kb deletion in 48% of the alleles of the examined Dutch patients. Furthermore, we screened for mutations in the CTNS gene of Dutch patients by direct sequencing of the different exons.

Next to cystine determination in polymorphic nuclear cells, molecular genetic analysis enables early diagnosis which is critical for the proper treatment of this disease.

Program Nr: 1319 from the 1999 ASHG Annual Meeting

Identification of LDL-R independent lysosomal targeting of cholesteryl esters (CE) and triglycerides (TG) using lysosomal acid lipase gene targeted mice. *M. Heur[1], H. Du[1], J. Mishra[1], D. Witte[2], G.A. Grabowski[1]*. 1) Div Human Genetics, Children's Hosp Res Fdn, Cincinnati, OH; 2) Div Pediatric Pathology, Children's Hosp Res Fdn, Cincinnati, OH.

Understanding regulation of cholesterol metabolism is essential due to the myriad of physiological functions of cholesterol and because hypercholesterolemia is the major risk factor for heart disease. Dietary CE, an important participant in regulation of cellular cholesterol metabolism, are delivered to the lysosomes where they are cleaved by lysosomal acid lipase (LAL) into cholesterol and free fatty acid. Cholesterol then exits the lysosome and participates in various cellular processes including regulation of cellular cholesterol metabolism. The objective of this study was to test the hypothesis that the low-density lipoprotein receptor (LDL-R) pathway is the major route through which dietary CE and TG are trafficked to the lysosomes. LAL knockout mice (LAL -/-) were bred to LDL-R knockout mice (LDL-R -/-) to produce offspring lacking both LAL and LDL-R (LAL -/-, LDL-R -/-). LAL -/- mice display massive hepatosplenomegaly secondary to CE and TG storage whereas LDL-R -/- mice appear normal physiologically and with respect to their serum lipid profiles. The elimination of LDL-R in LAL -/- mice would be expected to drastically reduce the hepatomegaly of LAL -/- mice. However, LAL -/-, LDL-R -/- mice had hepatosplenomegaly similar to LAL -/- mice. LAL -/-, LDL-R -/- total plasma cholesterol level was 60.37 mg/dL; this was similar to those of LAL -/- and LDL-R -/- mice, (54.43 mg/dL and 87.16 mg/dL, respectively). Total liver cholesterol levels in LAL -/-, LDL-R -/- and LAL -/- mice were similar while that of the LDL-R -/- mouse was about 10 fold lower than that in the LAL -/- mouse. The total liver cholesterol level of LAL -/-, LDL-R -/- mouse was 71.01 mg/mg while those of LAL -/- and LDL-R -/- mice were 109.11 mg/mg and 11.89 mg/mg, respectively. These data suggest a major role for alternate pathways in lysosomal delivery of dietary lipids in the absence of the LDL receptor.

Program Nr: 1320 from the 1999 ASHG Annual Meeting

Mutation Analysis in Succinic Semialdehyde Dehydrogenase (SSADH) deficiency (4-Hydroxybutyric Aciduria).

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SSADH deficiency is a rare heritable defect in the GABA degradative pathway leading to 4-hydroxybutyric (4-HB) aciduria; pathophysiology may relate to accumulated GABA, 4-HB acid, or synergism between both. Despite fairly consistent enzymatic and metabolic findings, the clinical phenotype featuring mainly nonspecific neurologic anomalies is highly heterogeneous. We undertook molecular analysis in cells derived from affected patients and performed mutation screening in samples obtained from 25 patients (21 families) using combined RT-PCR/genomic PCR and sequence analysis. The results were: three RNA splicing mutations (loss of exon 8; loss of exon 9, and a splicing mutation resulting in skipping of the first 14 bp of exon 4); one insertion (AG insertion at nt 457), one deletion (14 bp following nt 460); four nonsense mutations (Y128X, W204X, R412X and R514X), and 8 missense mutations (P182L, C223Y, T233M, A237S, G268E, P382L, G409D and G533R). This represents 21 alleles/18 mutations and adds substantively to our previous report of two exon-skipping mutations (AJHG 63, 399 (1998)). With the exception of the P182L, C223Y and G533R alleles, the mutations represented highly conserved residues in other (putative) SSADH proteins and other human aldehyde dehydrogenases. Thus far our analysis provides no clear insight into genotype/phenotype correlations, and indicate that most mutations are private within the families studied.

Program Nr: 1321 from the 1999 ASHG Annual Meeting

MUTATION ANALYSIS ON KOREAN PATIENTS WITH CITRULLINEMIA. *K.M. Hong[1], S.H. Hahn[2], M.K. Paik[1]*. 1) Department of Biochemistry, Wonkwang University College of Medicine, Iksan, Jeon-Buk, 570-749, Korea; 2) Departement of Pedriatics, Ajou University School of Medicine, Suwon, 442-749, Korea.

Citrullinemia is an autosomal recessive disease resulting from mutations in argininosuccinate synthetase (ASS) gene. In order to identify the frequent mutations in Korean patients with citrullinemia, studies on two patients were initially undertaken. The mRNAs were prepared from their fibroblasts, and the ASS cDNAs were amplified, cloned and sequenced. Both of them showed exon 7 deletion in their mRNAs resulted from splicing mutation previously reported as IVS-6^{-2} (a transition of A to G at the second nucleotide position within the 3' splice cleavage site of intron 6). This finding was confirmed by gDNA sequencing of the amplified product including the exon 7 and the surrounding partial intron sequences. Frequent IVS-6^{-2} mutation was reported in Japanese patients with citrullinemia, but not in Caucassian patients who showed extreme mutational heterogeneity. Although very limited number of patients were evaluated in this study, IVS-6^{-2} mutation might also be a frequent mutation in Korean patients suggesting possible founder effect. A point mutation in exon 13 and a novel insertional mutation in exon 15 were also identified in mRNAs of the patients. It appears that citrullinemia in Korean patients would be resulted from one common mutant allele (IVS-6^{-2}) and other rare alleles.

Program Nr: 1322 from the 1999 ASHG Annual Meeting

Isolation of novel genes involved in early stage of ectopic ossification by means of differential display method using *ttw* (tiptoe walking), a mouse model of ectopic ossification. *S. Ikegawa[1], Y. Koshizuka[1,2], M. Sano[1,3], K. Nakamura[2], A. Nagano[3], Y. Nakamura[1]*. 1) Laboratory of Genome Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan; 2) Department of Orthopaedic Surgery, Faculty of Medicine, The University of Tokyo, Tokyo, Japan; 3) Department of Orthopaedic Surgery, Hamamatsu University Scool of Medicine, Hamamatsu, Japan.

Ectopic ossification (bone formation at ectopic sites in the body) is often a severe complication of joint arthroplasty, neurologic trauma, and muscle injury; Ectopic ossification causes serious clinical conditions including OPLL (ossification of the posterior longitudinal ligament of the spine) and fibrodysplasia ossificans progressiva. Its pathogenesis, however, remains unclear. A naturally-occurring mouse mutant, *ttw* (tiptoe walking) is an excellent model for ectopic ossification. The mouse exhibits ossification of ligaments of the extremities and the spines, tendons and cartilages. The ossification of the spinal ligaments are radiographically and histologically very similar to that of human OPLL. We previously reported that the *ttw* phenotype is caused by a nonsense mutation in a gene encoding nucleotide pyrophosphatase, a membrane-bound ectoenzyme implicated in phosphate-pyrophosphate metabolism. To clarify the pathological mechanism of ectopic ossification, we examined the effect of dietary calcium and phosphate to the *ttw* phenotype, and found a high phosphate diet markedly accelerated the ectopic ossification of *ttw*. To isolate genes involved in this rapid and severe ectopic ossification, we investigated the gene expression in this model using differential display method. We have so far identified several novel genes which exhibited increased/decreased expression according to the diet, and characterized them. Our identification of the novel genes would give novel insights into mechanism of calcified tissue metabolism and ectopic ossification as well as the etiology of human OPLL.

Program Nr: 1323 from the 1999 ASHG Annual Meeting

Primary carnitine deficiency (PCD) as a novel cause of hereditary recurrent myoglobinuria. *F. Invernizzi, B. Garavaglia, M. Mora, C. Antozzi, F. Taroni.* Istituto Nazionale Neurologico C. Besta, Milan, Italy.

Common metabolic causes of paroxysmal myoglobinuria include enzyme defects of glycogen metabolism as well as defects of mitochondrial enzymes (most commonly CPT2) of long-chain fatty-acid oxidation. Although carnitine is an essential cofactor for long-chain fatty-acid oxidation, primary carnitine deficiency (PCD) has never been associated with pure recurrent myoglobinuria. Usually, it is a life-threatening disorder characterized by progressive cardiomyopathy and skeletal myopathy and/or hypoketotic hypoglycaemia and liver failure. We describe one new patient with exercise-induced myoglobinuria, no glycogen or lipid accumulation in skeletal muscle, normal activities of CPT2 and glycolytic enzymes, and normal profiles of urinary organic acids and plasma free fatty acids. The proband was a 22-year-old woman who had manifested recurrent episodes of paroxysmal myoglobinuria and muscle weakness since the age of 15y. There was no sign of cardiomyopathy, as documented by echocardiography. Plasma total carnitine was 10.5 mmol/l (n.v. 45±3). [^{3}H]L-carnitine uptake in fibroblasts was reduced to 25% of the control mean. After a few months of daily administration (3-6 g) of L-carnitine, treatment appeared to be beneficial. Muscular strength improved and metabolic attacks significantly reduced. A second muscle biopsy performed after 5 months of treatment demonstrated normalization of total L-carnitine levels. In conclusion, our findings indicate that PCD should always be considered as a cause of recurrent myoglobinuria even in the absence of lipid accumulation in muscle. Molecular analysis of the recently discovered PCD gene (*OCTN2*) is in progress, in order to clarify the molecular defect which underlies this unusual phenotype. (Partly supported by a Telethon-Italia grant to F.T.).

Program Nr: 1324 from the 1999 ASHG Annual Meeting

Liver transplantation for methyl malonic acidopathy (MMA disease) is not curative: cerebral production of methyl malonic acid (MMA) is significant in the pathogenesis of disease. *P. Kaplan, A.M. Mazur, M. Palmieri, G.T. Berry.* Biochemical Genetics,Childrens Hospital of philadelphia, Univ of Pennsylvania, PA 19104-3499.

Treatment of organic acidopathies, mainly nutritional, has variable, sub-optimal outcomes. Ideal treatment would entail enzyme replacement by enzyme infusion, liver or bone marrow transplantation [Tx], or gene therapy. Orthotopic liver Tx was done in a 19 month boy with mut0 MMA, at his parents request. Blood, cerebrospinal fluid[CSF] and urine levels of MMA were elevated 2455 fold, 1642 fold[1 measurement] & 200-1200 fold respectively pre-Tx.Post-Tx,these levels decreased by ~50% in blood and urine, but only by 10-20% in 5 of 6 CSF analyses, and remain elevated in all fluids 28mos post-Tx. Average CSF:plasma MMA was 2:1 pre-Tx and 4:1 post-Tx. Dietary protein 1.2gm-2gm/kg/day is tolerated without acidosis or bicarbonate therapy. Truncal & axial tremors developed post-Tx, were attributed to tacrolimus therapy, and resolved after many months. Growth, neurologic status and renal function continue to be normal. Metabolic decompensation has not occurred despite several infections and stresses. Conclusions: Liver Tx does not cure or significantly ameliorate the disease in mut0 MMA. This is the first longitudinal study of MMA disease comparing levels in CSF with other body fluids pre - and post-Tx. MMA production in brain appears to contribute significantly to the burden of disease but does not affect neurologic or cognitive function significantly in the proband.

Program Nr: 1325 from the 1999 ASHG Annual Meeting

A novel nonsense mutation (G5920A) in the mtDNA COX I gene: another cause of myopathy with myoglobinuria. *C.L. karadimas[1], P. Greenstein[2], J.T. Joseph[2], E. Bonilla[1], K. Tanji[1], S. Shanske[1], C.M. Sue[1], S. DiMauro[1]*. 1) Department of Neurology, Columbia University, College of Physicians & Surgeons, New York, NY; 2) Deparments of Neurology and Pathology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA.

A 34-year-old man had life-long intolerance to sustained exercise, intermittent weakness, and recurrent myoglobinuria. There was no evidence of multisystem disorder, and family history was negative for neuromuscular diseases. Muscle biopsy showed numerous COX-negative ragged red fibers. Electron microscopy confirmed the presence of overabundant and structurally abnormal mitochondria. Analysis of respiratory chain enzymes in muscle homogenate revealed a marked decrease of COX activity (13% of normal), while the activities of other complexes were normal. Direct sequencing of the mitochondrial genes encoding COX subunits revealed a G5920A heteroplasmic mutation in the COX I gene, changing a Trp to a stop codon. No other potential pathogenic nucleotide changes were detected nor did we find this mutation by PCR/RFLP analysis in 100 control muscles. This mutation predicts the loss of almost the entire COX I protein, thus easily explaining the clinical, morphological, and biochemical phenotype of the patient. (Supported by NIH grant P01 HD32062 and by a grant from the Muscular Dystrophy Association).

Program Nr: 1326 from the 1999 ASHG Annual Meeting

Glucose-6-phosphate dehydrogenase deficiency in Wilson's disease patient aggravates copper induced toxicity: Possible role of depleted levels of glutathione. *G. Kaur[1], R. Prasad[2], B.R. Thapa[3]*. 1) Deptt. of Physiology, Govt. Medical College,Chandigarh, India; 2) Deptt. of Biochemistry, Postgraduate Institute of Medical Education and Research,Chandigarh, India; 3) Deptt. of Gastroenterology, PGIMER, Chandigarh,India.

A five and a half years male child of Wilson's disease was admitted at early onset with both hepatological and neurological manifestations as well as glucose-6-phosphate dehydrogenase deficiency. Neurological manifestations have been reported mostly after 12 years of age. This study is of utmost importance from molecular basis of pathophysiology and clinical point of view. Work-up for Wilson's disease showed urinary copper:313mg/24 h, post-D-penicillamine urinary copper:459mg/24 h, and serum ceruloplasmin:3.6 mg/dl. In this case, plasma GSH level was found to be 0.64mmol GSH conjugated/g Hb, which is markedly lower than age-matched control (1.2mmol/g Hb). In this patient superoxide dismutase activity in RBC hemolysate was 0.352 units/g Hb, which was lower than that in the age-matched control (0.611 units/g Hb). Membrane lipid peroxidation was assessed by measuring malondialdehyde (MDA) in RBC hemolysate and found to be 0.069mmol MDA/g Hb. It was significantly higher than the normal level (0.029mmol MDA/g Hb). Thus, as a result the potential prooxidant action of excess copper and low level of glutathione could be significant determinants for cell susceptibility to the deleterious action of free copper which may lead to early onset of disease with both hepatological and neurological manifestations.

Program Nr: 1327 from the 1999 ASHG Annual Meeting

Rapid noninvasive diagnosis of nonketotic hyperglycinemia (NKH) by proton MR spectroscopy in a Korean newborn patient with NKH. *E. Kim[1], K. Kim[1], S. Pi[1], H. Yoo[2]*. 1) Division of Neonatology, Asan Medical Center, Seoul, Korea; 2) Medical Genetics Clinic & Laboratory, Asan Medical Center, Seoul, Korea.

Nonketotic hyperglycinemia (NKH) is an autosomal recessive inherited metabolic disorder caused by the defects in the glycine cleavage system(GCS; EC2.1.2.10), a multienzyme system that consists of four individual components. The NKH is clinically characterized by rapidly progressing neurologic symptoms, such as muscular hypotonia, seizures, apneic attacks, and lethargy or coma in the neonatal period. Increased glycine concentrations in plasma, urine, and especially in cerebrospinal fluid (CSF) without ketosis are key biochemical features. A Korean newborn patient who is the first normal full term product from healthy parents of non-consanguinous marriage, developed a sudden apneic attack at 28 hours after birth with frequent hiccups. Initial laboratory work-up revealed no metabolic acidosis, no hypoglycemia, no hyperammonemia, no ketosis, and no abnormal neurosonographic findings. However, electroencephalogram showed a burst-suppression pattern. Based on these clinical features, presumptive diagnosis of NKH has been made. By using localized proton MR spectroscopy, we identified a pathologic peak signal of glycine in the parietooccipital white matter and in the basal ganglia before the result of the ratio of CSF glycine concentration to that of plasma was available, which turned out to be 0.13. The follow-up proton MR spectroscopy was performed with treatment. The proton MR spectroscopy can be utilized as a non-invasive adjunctive diagnostic or monitoring tool for NKH patients.

Program Nr: 1328 from the 1999 ASHG Annual Meeting

Genetic, biochemical and pathological studies of four Japanese patients with neonatal Gaucher disease. *M. Kobayashi, H. Ida, Y. Eto.* Dept Pediatrics, Jikei Univ, Minato-ku, Tokyo, Japan.

Gaucher disease (GD) is caused by a deficiency of glucocerebrosidase, resulting in strorage of glucocerebroside (GC) in reticuloendothelial system such as liver, spleen and bone marrow. The clinial hallmark of GD is a variable phenotypic expression. There are three clinical phenotype-type 1 (non-neuronopathic form), type 2 (acute neuronpathic form) and type 3 subacute neuronopathic form). This classification is based on the onset and severity of neurological symptoms. The phenotypic expression of type 2 GD was thought to be sterotypic in presentation, with infantile onset, progressive neurological deterioration and death by 2 years old. We identified 85 Japanese patients with GD until 1998. Of 85 patients 15 patients were classified into type 2 GD. Of 15 cases with type 2 four cases manifested none-immune hydrops fetalis and they died within 2 months. Few biochemical and pathological studies have not been performed in this clinically unique patients. Up to date only three reports performed mutation analysis of lethal type 2 GD, demonstrating that two were homozygous for RecNciI and one patient was genotyped as compound heterozygote for a novel recombinant allele and a rare splice junction mutation. We here described pathological, biochemical and genetic studies of four Japanese patints with neonatal GD. The massive accumulation of GC was found not only in liver and spleen but in lung, kidney, brain and thymus in all cases. The pathological findings were characterized by destruction of tissues rather than accumulation of Gaucher cells. The genotypes of three patients examined were 1447-1466 del 20 ins TG/L444R, 1447-1466 del 20 ins TG/? and IVS2+1/?. These data suggest that Japanese neonatal GD shows quite unique genetic, pathological and biochemical findings.

Program Nr: 1329 from the 1999 ASHG Annual Meeting

Homozygosity mapping indicates high prevalence of complementation group A in Tunisian Fanconi anemia patients. *S. Abdelhak[1], C. Bouchlaka[1], H. Ben Abid[2], M. Frikha[3], S. Nabli[4], A. Saad[4], F. Fakhfakh[3], H. Ayadi[3], A. Hafsia[2], H. Joenje[5], K. Dellagi[1], For the Tunisian Fanconi anemia Study Group[1,2,3,4]*. 1) Immunology Dpt, Institut Pasteur de Tunis, Tunis, Tunisia; 2) Hematology Dpt, Aziza Othmana Hospital, Tunis, Tunisia; 3) Hematology Dpt, Habib Bourguiba Hospital, Sfax, Tunisia; 4) Hematology Dpt, Farhat Hached Hospital, Sousse, Tunisia; 5) Clinical Genetics and Human Genetics Dpt, Free University Medical Center, Amsterdam,The Netherlands.

Fanconi anemia (FA) is an autosomal recessive rare disorder characterized by bone marrow failure, multiple congenital malformations and an increased risk to develop cancer due to chromosomal instability. At least eight complementation groups have been defined, five of which have been mapped to human chromosomes. FANCA, the gene responsible for the most prevalent complementation group, has been identified and mapped to chromosome 16q24.3. In the present study, six polymorphic markers overlapping the FANCA region, have been used to genotype 15 FA families from different geographical areas of Tunisia. Homozygosity mapping showed that in 12 of these families the patients were homozygous by descent for these markers, suggesting an assignment to complementation group A. Two families were not informative. One patient born to consanguineous parents was heterozygous for all the markers overlapping FANCA region as well as for markers of FANCC, G and D genes. This family may thus belong to group B, E, F or H or to a new complementation group. Our results are compatible with a high prevalence of complementation group A in Tunisia.

Program Nr: 1330 from the 1999 ASHG Annual Meeting

A General Family Test of Linkage Disequilibrium for Quantitative Traits. *G.R. Abecasis, L.R. Cardon, W.O.C. Cookson.* Wellcome Trust Center for Human Genetics, University of Oxford, OX3 7BN, United Kingdom.

High-resolution mapping is an important step in the identification of complex disease genes. In outbred populations, linkage disequilibrium is expected to operate over short distances and could provide a powerful fine-mapping tool. Here we build upon recently developed methods for linkage disequilibrium mapping of quantitative traits to construct a general approach that can accommodate families of any size, with or without parental information. Variance components are used to construct a test that utilizes information from all individuals but is not biased in the presence of linkage or familiality. Family data allows for the construction of an expected genotype for every non-founder, and we show that focusing on orthogonal deviates from this expectation makes it possible to distinguish association due to linkage disequilibrium from other types of association. For smaller families, a permutation test is described for situations where multivariate normality is violated and maximum likelihood estimates of the variance components are unreliable.

Simulation studies are used to investigate power and error rates of these approaches, and to highlight situations in which violations of multivariate normality assumptions warrant the permutation test. The relationship between power and the level of linkage disequilibrium for this test suggests that the method is well suited to the analysis of dense maps. The relationship between power and family structure is investigated and these results are applicable to study design in complex disease, especially for late-onset conditions where parents are usually not available. When parental genotypes are available, power does not depend greatly on the number of offspring in each family but only the total sample size. Power decreases when founder genotypes are not available, but the loss in power is negligible for larger families. Finally, we identify some situations where the number of individuals to genotype can be reduced with little loss in power.

Program Nr: 1331 from the 1999 ASHG Annual Meeting

Disequilibrium mapping of quantitative trait loci in the Hutterites. *M.A. Abney[1], M.S. McPeek[2], C. Ober[1].* 1) Dept Human Genetics, Univ Chicago, Chicago, IL; 2) Dept of Statistics, Univ of Chicago, Chicago, IL.

Founder populations facilitate the search for genes that underlie complex phenotypes because of the relatively small number of independent genomes and recent origins. However, the large, complex genealogies that are characteristic of these populations pose considerable analytical and computational challenges. We have developed disequilibrium-based methods to map quantitative trait loci in the Hutterites. To detect recessive traits, we calculate at each locus a homozygosity-by-descent (HBD) statistic, which takes into account the exact known relationship between the parents of each subject, the genotype at the marker locus and at each flanking locus, and the distance to flanking loci. We consider both regression and normal mixture models based on this statistic, adjusting for covariates, and including polygenic components of variance. We also consider dominant and additive trait models. We use simulation to assess the statistical properties of the methods and determine significance. We apply the method to various quantitative traits in the Hutterites.

Program Nr: 1332 from the 1999 ASHG Annual Meeting

Magnitude of Type I Error of Heterogeneity Lod Score for Two-point Analysis. *P.C. Abreu*[1], *D.A. Greenberg*[2], *S.E. Hodge*[1,3,4]. 1) Div. of Biostatistics, Columbia Univ, New York,NY; 2) Dept. of Psychiatry, Mount Sinai Medical Ctr, New York,NY; 3) Dept. of Psychiatry, Columbia Univ,New York, NY; 4) NYSPI, New York, NY.

Linkage heterogeneity is a major confounding factor in linkage analysis. The classical distribution theory of the log likelihood ratio test statistic does not hold when testing for linkage and heterogeneity, i.e., mixture alternatives. We used computer simulations to explore the mixture problem in the genetic linkage context. We investigated the type I error of the heterogeneity lod score (HLOD), i.e., maximizing the lod score over both recombination fraction q and admixture parameter a. We examined two situations: (a) assuming a single genetic model and (b) maximizing HLOD over dominance models (dominant vs. recessive). We generated data sets of both phase-known and phase-unknown families under autosomal dominant inheritance with full penetrance. The asymptotic distributions of the linkage test statistics are a mixture of three c^2 distributions in the phase-unknown case, and of two c^2 distributions in the phase-known case.

For part (a) significance levels for phase-unknown families did not increase when maximizing w.r.t q and a compared to significance levels when maximizing only w.r.t q. For the phase-known case, the significance levels were increased somewhat when maximizing over both a and q compared to only w.r.t q, but the increase did not reach an additional degree of freedom. In part (b), when maximizing HLOD over q, a, and dominance model, the significance levels for the phase-unknown case were essentially the same as when maximizing only w.r.t q and dominance model, but not a. The significance levels for phase-known families are slightly higher than for phase-unknown. Because the correction for maximizing over dominance model (Hodge et al, 1997) is already very conservative, we recommend no additional increase in the correction of type I error for phase-known families. That is, the inflation of type I error when maximizing the max HLOD over dominance model can be corrected by increasing the lod score cutoff from a nominal value 3.0 to a cutoff value of 3.3, the same correction as for simply maximizing the max lod score over dominance model.

Program Nr: 1333 from the 1999 ASHG Annual Meeting

Evaluation of the CMT1A locus in African-American CMT patients. *M. Ahearn[1], L. Baumbach[1], E. Perera[1], K. Jackson[2], T. Bird[3], W.A. Bradley[1]*. 1) Univ. of Miami Medical School, Miami, FL; 2) Tulane Univ. Medical School, New Orleans, LA; 3) VA Medical Center, Seattle, WA.

The vast majority of reported patients with severe, dysmyelinating Charcot-Marie-Tooth Disease (CMT1) are of Caucasian ancestry. African-American (AA) CMT1 patients are greatly underrepresented clinically, as well as in published molecular studies, raising clinically interesting questions regarding possible ethnic-specific genetic differences which may modify disease phenotypes. We report our evaluation of the CMT1A locus in seven AA families. These studies have focused on identif ication of disease causing mutations in the 17p11.2 region in these families, as well the prevalence of a *pmp22* intron 3 EcoR1 polymorphism we previously reported (AJHG 61:A343,1997).

We have completed analysis of four of the seven probands, with the remaining patient and family studies nearing completion. Patient 1, Cuban ethnicity with African-American ancestry, has the 1.5 mB CMT1A duplication; Patient 2, who displays a Dejerrine-Sottas phenotype, has a novel *pmp22* point mutation; and it appears that patients 3 and 4 lack the CMT1A duplication and are being tested for *pmp22* mutations. Each of these patients also had the EcoR1 polymorphism, in comparison to a 35% frequency in African- American control samples. This polymorphism has never been reported in Caucasian CMT1A patients. Interestingly, patient 1, affected with CMT since early childhood, was significantly more symptomatic at the same age than her affected mother, who has the duplication, but does not have the EcoR1 polymorphism. Patients 3 and 4 are members of unrelated families which have had multiple members affected by their early thirties. Identification of the disease-causing mutations, and analysis of the segregation of the *pmp22* polymorphism in these kindreds, is being completed.

These investigations represent the first analyses of the CMT1A locus in African-American CMT patients and address a possible association of the apparently AA-specific *pmp22* polymorphic variant with disease presentation.

Program Nr: 1334 from the 1999 ASHG Annual Meeting

Genome scan in Finnish families with keratoconus. *T. Alitalo[1,2,3], H. Tyynismaa[1,2], M. Uusitalo[2], P. Sistonen[4], A. Dammert[5], T. Latvala[5].* 1) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 2) The Folkhalsan Institute of Genetics, Department of Molecular Genetics, Helsinki, Finland; 3) Depts. of Obstetrics and Gynecology, Helsinki University Central Hospital ,Helsinki,Finland; 4) Finnish Red Cross, Blood Transfusion Center, Helsinki, Finland; 5) Dept. of Ophthalmology, University of Oulu, Finland.

Keratoconus is a bilateral noninflammatory thinning disorder of the cornea with an incidence of approximately 1/2000 in the general population. Histopathological findings include stromal thinning, iron deposition in the epithelial basement membrane, and breaks in Bowman's layer. The most common treatment for keratoconus is contact lenses, but when contact lenses fail, corneal transplant is the best surgical option. Pedigrees with keratoconus in both sexes in two or three successive generations suggest autosomal dominant inheritance. In general, the dominant pattern is irregular, affecting approximately 1 in 10 blood relatives instead of the expected 1 in 2, implying variable expression or incomplete penetrance. We have collected blood samples from 22 Finnish keratoconus families with autosomal dominant pattern of inheritance and confirmed the diagnosis by re-examining the eyes of the family members. All the families originate from a restricted geographical region. To localize the keratoconus gene, we have started a genome wide scan with a panel of 369 markers with an average spacing of 10 cM. Initial analysis of the genotype data using GENEHUNTER revealed one region with suggestive linkage. We have also performed SSCP analyses, and excluded TIMP2 as the causative gene in keratoconus.

Program Nr: 1335 from the 1999 ASHG Annual Meeting

Investigation of HLA-DR effects on susceptibility to Rheumatoid Arthritis (RA) using family-based association and affected sib-pair linkage methods in a large sample of European families. *H. Alves[1], M. Martinez[2], D. Charron[3], V. Lepage[3], C. Stavropoulos[4], F. Tores[2], R. Westhovens[4], F. Cornélis[5], European Consortium E.C.R.A.F.[4].*
1) Department of Rheumatology, S Joao Hospital, Porto, Portugal; 2) INSERM U358, Paris, France; 3) Laboratoire D'Histocompatibilité, Hopital St Louis, Paris, France; 4) EEC-BIOMED2; 5) Univeristé Paris 7-Denis Diderot, France.

The mode of inheritance of RA is not known, but susceptibility to RA is associated to HLA-DR. The shared-epitope (SE) hypothesis has been postulated to support direct involvement in susceptibility of specific HLA-DR alleles. Others have suggested that DR effects may vary with epidemiological or clinical factors as gender, severity and onset of the disease. A complex genetic mechanism of the HLA-DR gene and genetic heterogeneity can explain these conflicting results. Small sample sizes and population stratification problems have also entangled these investigations. To date, the ECRAF consortium (Cornelis et al., 1998) is one of the largest collection of triads (one RA sib and parents, n=160) and ASP (at least 2 RA sibs, n=361) ever collected. Linkage disequilibrium to HLA-DR SE alleles (SE=0101, 0403, 0404, 0405, 0408, 1001, X= other alleles) was tested using family-based statistics in the triad sample. Probands from the different groups (France, n=84; Belgium, n=23; Spain, n=20; Italy, n=19, Portugal, n=14) have similar gender ratios, mean age of onset and clinical characteristics. TDT test was highly significant ($p=310^{-5}$). All SE alleles were significantly associated to RA except 0404 and 0408 alleles (p>5%). No significant differences for the parental origin of the transmitted alleles were found. Investigations of SE distributions by epidemiological and clinical characteristics of probands showed significant differences for the presence/absence of rheumatoid factors: SE alleles are more frequent in RF+ than in RF- probands (p=0.002). Linkage to the HLA-DR region (tested markers are D6S276, Tnfa and DR) was confirmed in our ASP sample (MLS=11.71). However, simple DR gene effects, as the SE hypothesis, can not explain our ASP linkage data.

Program Nr: 1336 from the 1999 ASHG Annual Meeting

A new locus for autosomal dominant hearing loss DFNA28 mapped to chomosome 8q22. *D.W. Anderson[1], A.J. Griffith[1], S. Rudy[1], T. San Agustin[2], T.B. Friedman[1], R.J. Morell[1].* 1) Laboratory of Molecular Genetics, NIDCD, NIH, Rockville, MD; 2) NIDRR, U.S. Dept. of Education, Washington, DC.

We have ascertained a large American family with an autosomal dominant form of nonsyndromic sensorineural hearing loss. The hearing loss is postlingual, with an onset as early as seven years of age, and gradual progression to moderate to severe levels by the fifth decade. The audiometric pattern is variable, with predominant impairment of high frequencies in some affected individuals and middle frequencies in others. Speech discrimination scores are normal, indicating that the loss is cochlear in origin.

Twenty-four individuals from four generations, including 9 affected individuals, were initially screened with STR markers linked to the reported DFNA and DFNB loci. These loci were all eliminated from consideration on the basis of two point linkage analysis. A genome wide scan using the Weber 8 panels was performed. Linkage to marker GAAT1A4 , at 8q22, was detected with a LOD (z) = 4.86 at q = 0. Haplotype analysis using markers flanking GAAT1A4 defined a 4 cM critical region between markers D8S546 and D8S545. This region excludes two loci for syndromic deafness: Branchio-Oto-Renal syndrome (BOR) and Hereditary Motor Sensory Neuropathy-Lom (HMSNL). There are no nonsyndromic deafness loci mapped to chromosome 8q22. Therefore, this family represents a new deafness locus, DFNA 28.

Program Nr: 1337 from the 1999 ASHG Annual Meeting

Microsatellite marker associations around the HLA-C region in Finnish psoriasis patients and controls. *K. Asumalahti[1], T. Laitinen[1], M. Lokki[2], H. Mannila[3], U. Saarialho-Kere[4], R. Itkonen-Vatjus[5], J. Kere[1].* 1) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 2) Finnish Red Cross, Transfusion Service, Tissue-typing Laboratory, Helsinki; 3) Department of Computer Science, University of Helsinki; 4) Department of Skin Diseases, University Hospital of Helsinki; 5) Department of Skin Diseases, Central Hospital of Kainuu, Kajaani.

Psoriasis is a chronic skin disease characterized by hyperproliferation of epidermal keratinocytes and mononuclear cell mediated inflammation affecting 2-3% of Caucasoid population. In recent years, several candidate loci for psoriasis have been reported. The oldest and still strongest evidence has been found in HLA association studies and the HLA-Cw*0602 allele has been suggested as a major locus for psoriasis in many populations with the relative risk (RR) of 3-4. We verified the same allele association using SSP-PCR method in 142 psoriasis patients from the central eastern part of Finland (the Kainuu province). The Kainuu population is genetically isolated with a strong founder effect. Thirty-seven percent (52/142) of psoriasis patients were HLA-Cw*0602 positive compared to 9.6% (11/115) of healthy family based controls (RR 3.9). The relative risk was even higher (5.1) among the type I psoriasis patients (26/53, 49%). To estimate our power to identify a susceptibility allele in psoriasis by haplotype analysis, we studied four polymorphic microsatellite markers spanning 5cM across the HLA-C locus. The HLA-Cw*0602 allele was found in 35 unrelated chromosomes among the patients. The initial results showed that markers on both sides of the HLA-Cw*0602 identified altogether 15 different 2 marker haplotypes, but two of the haplotypes identified 60% (21/35) of the susceptibility chromosomes.

Program Nr: 1338 from the 1999 ASHG Annual Meeting

Linkage of systolic blood pressure to D21S1440 in Mexican Americans. *L.D. Atwood[1], P.B. Samollow[2], J.E. Hixson[2], M.P. Stern[3], J.W. MacCluer[2].* 1) Epidemiology, University of Minnesota, Minneapolis; 2) Genetics, Southwest Foundation for Biomedical Research, San Antonio; 3) Medicine, University of Texas Health Science Center, San Antonio.

We performed a genome scan of systolic blood pressure (SBP) on approximately 441 individuals in 10 randomly ascertained families of the San Antonio Family Heart Study. We defined a likelihood-based single-locus Mendelian model incorporating sex-specific and genotype-specific effects of age (linear and quadratic), BMI, and diastolic blood pressure (DBP) as covariates. A threshold correction was used for individuals on high blood pressure medication. Randomization of individuals across pedigrees showed that the effect of pedigree structure was significant (p=0.01), and a likelihood ratio test rejected the polygenic model (p=2.36E-64). Together, these two results indicate that SBP is oligogenic. Sex-specific and genotype-specific effects of age (p=6.12E-14 and p=2.58E-36) and BMI (p=3.39E-4 and p=8.04E-4) were highly significant, indicating that SBP has significant interaction effects. DBP had a very strong linear effect (p=3.91E-118), but did not have sex-specific or genotype-specific effects (p=.764 and p=.058).

Using model parameters that gave the global maximum likelihood we performed two-point linkage analysis using 401 highly polymorphic markers. SBP showed suggestive linkage to only two markers, *D18S844* (Z=2.09, q=0.11) and *D21S1440* (Z=2.82, q=0.00). For an oligogenic trait a combined segregation and linkage analysis (CSL) may be able to isolate a single locus signal more effectively than a fixed model analysis. Therefore, for these two markers we performed a CSL analysis in which both the segregation and recombination parameters for a two-locus model were maximized simultaneously. This model was maximized twice, once with the recombination fraction free and once with it fixed at 0.5. The resulting CSL lodscores showed that *D18S844* remained suggestive (Z=2.01, q=0.11). However, *D21S1440* reached significance (Z=3.24, q=0.00). A examination of GeneMap98 showed no obvious candidate genes near either marker.

Program Nr: 1339 from the 1999 ASHG Annual Meeting

Meta-analysis for interpreting results from multiple genome scans. *J.A. Badner, E.S. Gershon.* Dept Psychiatry, Univ Chicago, Chicago, IL.

Lander and Kruglyak gave guidelines for interpreting linkage results based on estimating how often a particular threshold for significance would be exceeded by chance in a single genome scan. A criterion for significant linkage was recommended to be a threshold that would be exceeded 0.05 times by chance in a single genome scan. These criteria do not always enable interpretation of the results of multiple genome scans of a genetic trait. In some cases, there may be several scans that show significant results within the same region but none of the individual results would meet criteria for significant linkage. In other cases, two or more studies exceed criteria for significant linkage but several other studies do not show nominally significant results with the same region. This may occur due to low power to detect genes of small effect using the criteria recommended by Lander and Kruglyak. One possibility is to combine the results of these studies. However, this is difficult to do in many instances. We propose a type of meta-analysis which involves combining p values across the studies. We make recommendations for criteria of linkage using this analysis. Comparisons of the power of this meta-analysis with Lander and Kruglyak criteria are presented. We apply this method of meta-analysis to the evidence for linkage of IDDM susceptibility loci.

Program Nr: 1340 from the 1999 ASHG Annual Meeting

Identification of and adjustment for genotyping errors in data on sibpairs when parental genotypes are unavailable. *M. Bahlo*[1], *K.W. Broman*[2], *T.P. Speed*[1,3]. 1) Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia; 2) Marshfield Medical Research Foundation, Marshfield, WI; 3) University of California, Berkeley.

In affected sibpair studies of late-onset diseases, such as prostate cancer, DNA samples for subjects' parents are often unavailable. In such situtations, genotyping errors cannot be detected by the usual methods, since all possible genotypes conform to Mendel's rules. We have studied two approaches to dealing with genotyping errors in such data, making use of multipoint marker information. First, we consider a likelihood ratio statistic to identify genotypes likely to be in error. Second, we calculate IBD probabilities given multipoint marker data using an incomplete penetrance function which allows for the presence of errors. We present the results of a simulation study comparing the power to detect a disease susceptibility gene when (a) genotyping errors are ignored, (b) genotypes likely to be in error are detected and removed, and (c) using an analytic method which allows for the presence of genotyping errors. We studied the effect of marker density, different error models, gene effect, sample size and error rate on the outcome. Genotyping errors are shown to erode the power to detect linkage. The effect may be ameliorated by use of methods to detect or allow for the presence of errors, though marker density must be quite high.

Program Nr: 1341 from the 1999 ASHG Annual Meeting

Mapping of human $GABA_A$ receptor subunit genes and generation of polymorphic markers. *M.E.S. Bailey, G.R. Riboldi Tunnicliffe, A. McDonnell, M. Ennis, J. Du, K.J. Johnson.* Division of Molecular Genetics, IBLS, Univ. of Glasgow, Glasgow, U.K.

$GABA_A$ and $GABA_C$ (g-aminobutyric acid types A and C) receptors mediate a significant proportion of fast inhibitory neurotransmission in the vertebrate central nervous system and retina. The receptors are pentameric assemblies of membrane-spanning subunits that enclose a chloride-selective, GABA-gated channel. The subunits share a common structure and have been classified in 7 subclasses (a - e, p, and r) based both on their relative degree of sequence similarity and on their pharmacology. Each of the 19 known mammalian subunits is encoded by a separate gene, all of which are members of the inhibitory branch of the ligand-gated ion channel (LGIC) gene superfamily. We have localised several of these subunit genes in detail, particularly those lying on human chromosome 4p, using linkage, radiation hybrid and STS/YAC mapping techniques. We have mapped *GABRR3* (the previously uncloned human orthologue of the rat r3 subunit gene) to human chromosome 3q. During these studies, we generated and characterised polymorphic, microsatellite markers for *GABRA2*, *GABRA4*, *GABRG2* and *GABRR1*. These will prove of use in linkage analysis of human neurological and psychiatric disorders and traits.

Most of the subunit genes in this family are arranged in 4 clusters on different chromosomes. Our human mapping data, along with existing data and the preliminary results of our comparative mapping study in *Fugu*, indicate that there has been a striking degree of conservation of gene order and orientation within these paralogous clusters. Phylogenetic analysis using PAUP has yielded a model for the evolution of this gene family that is largely consistent with the genomic localisations of its human members; this model lends support to the idea that two genomic duplication (tetraploidisation) events occurred early in chordate evolution. The role of regulatory and coding sequence variation in influencing human traits, and the possible links between gene cluster conservation and functional specialisation of the paralogous genes are just starting to be addressed.

Program Nr: 1342 from the 1999 ASHG Annual Meeting

Automated lane tracking and sizing of DNA fragments. *F.K. Bandukwala[1,2], M.W. Perlin[1,2,3]*. 1) Cybergenetics, Co., Pittsburgh, PA; 2) School of Computer Science, Carnegie Mellon University, Pittsburgh, PA; 3) Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA.

Human editing of DNA fragment data is a key bottleneck precluding the cost-effective automation of microsatellite and SNP genotyping data. When the data are derived from electrophoretic separation of fluorescently labeled markers on sequencing gels, the analysis tasks include tracking lanes and sizing DNA fragments. Fully automated lane tracking and sizing can be challenging in the presence of data artifacts, such as geometric peak and lane distortions. We have developed a pattern recognition module based on signal processing methods that implements this automation for our TrueAllele(TM) automated genotyping software.

Our method automatically extracts the lanes by first analyzing the largest size row of size standards (SS). Steps include deconvolution (for increased signal to noise), model function fitting (with two-dimensional Gaussian functions), and feature extraction (using shape parameters). Once the distributions of all the peaks in one SS row have been identified, the pattern is transferred to the next SS row by identifying matching geometric features in the next region. Matched features require consistency between intensity shape near the data peak, and consistency between geometric constraints. The peak data in SS row k are extracted by distorting the pattern of SS row (k-1), and obtaining a good fit between that pattern and strong candidate peaks in SS row k.

Our method automates the lane tracking and sizing of 96-lane ABI/377XL gels on Macintosh, Windows, and UNIX computers. On an iMAC computer, after an initial ten minute learning run, the processing time is reduced to about two minutes per gel. The program generates internal quality measures that can eliminate human review for the majority of gels, and direct user attention to problematic data regions on poorly-tracking gels.

Program Nr: 1343 from the 1999 ASHG Annual Meeting

The role of chemokine receptors in susceptibility to multiple sclerosis. *L.F. Barcellos[1], A.M. Schito[1], J.B. Rimmler[2], E. Vittinghoff[1], A. Shih[1], R. Lincoln[1], D.E. Goodkin[1], J.L. Haines[3], M.A. Pericak-Vance[2], S.L. Hauser[1], J.R. Oksenberg[1]*. 1) Dept. Neurology, Univ. California, San Francisco, CA; 2) Center for Human Genetics, Duke Univ., Durham, NC; 3) Program in Human Genetics, Vanderbilt Univ., Nashville, TN.

The etiology of multiple sclerosis (MS) pathogenesis is complex with involvement of both environmental and genetic influences. Recent results obtained from full genome screening identified several susceptibility regions, supporting a polygenic model for MS. Among these regions, evidence for weak linkage was observed at 3p/3cen suggesting the presence of a MS gene(s) of modest effect. Encoded here are two chemokine receptors, CCR5 and CCR2B. Clinical, experimental and epidemiological studies strongly suggest that both are potential MS candidate genes. We examined the ch.3p21-24 region in 125 MS families (322 total affecteds and 200 affected sibpairs) and performed genetic analyses of CCR5 and CCR2B loci and two nearby markers (D3S1289 and D3S1300) using both linkage and association-based tests. Modest linkage was observed for D3S1300 in the HLA-DR2 negative families only, 1.74(q=0.01) and 1.57(q=0.01), using both affected recessive or affected dominant models, respectively. Results obtained from ARP (SimIBD), sib-pair analyses (ASPEX), and association testing (sib-TDT) for each locus were not significant. However, significant effects on age of onset in patients were present for both gender and CCR5 phenotype (presence or absence of 32 bp deletion or D32) variables. Age of onset was approximately 3 years later in males compared to female MS patients (p=0.004) and approximately 3 years later in individuals with the CCR5D32 allele (p=0.018) after controlling for gender effects. This was not observed in a sample of 238 sporadic patients indicating that heterogeneity may exist between the two groups. Our results suggest that CCR5D32 may delay age of onset in familial MS. Polymorphisms within several chemokine receptors have recently been shown to significantly influence the risk of HIV infection and to delay progression. It will be of interest to examine the role of CCR5 in MS disease progression, CNS activity and severity.

Program Nr: 1344 from the 1999 ASHG Annual Meeting

Patterns of maternal transmission in asthma at chromosome 12q21.31. *K.C. Barnes[1], L.R. Freidhoff[1], R.A. Mathias[1], R.P. Naidu[2], R. Nickel[1], P.N. Levett[2], T.H. Beaty[1].* 1) Johns Hopkins University, Baltimore, MD; 2) University of West Indies, Barbados.

Asthma and stopy are referred to as multifactorial traits because disease expression is thought to be influenced by interactions between major and minor genes, and modulated by interacting non-genetic factors, such as environment. 'Developmental noise' (e.g., fetal-maternal environment) may interact with certain susceptible genotyopes, subsequently manifesting as diease. Epidemiologic data supports the joint effects of genetic susceptibility and parent-of-origin effect on the transmission of asthma, and several groups have reported evidence for linkage between maternal alleles and asthma or atopy at different loci, including chromosomes 4q, 11q, and 16q. We investigated the parent-of-origin effect on the transmission of asthma, allergis rhinitis (AR), and high serum total IgE (tIgE) in a set of 33 multiplex families from Barbados, ascertained through an affected offspring to determine: (1) the observed frequency and odds ratios (OR) of disease for the offspring of affected mothers compared to the offspring of affected fathers; and (2) preferential transmission of maternal or paternal alleles to affected probands using 22 chromosome 12q markers and transmission/disequilibrium testing (TDT). Offspring (asthma=130; AR=123; high tIgE=199) with an affected mother were more than twice as likely to be affected (asthma: OR, 2.7; 95% CI, 1.56-4.62; AR: OR, 2.1; 95% CI, 1.28-3.42; high tIgE: OR, 2.25; 95% CI, 1.46-3.45). Using the allele-by-allele TDT on 87 independent trios for asthma, we observed modest evidence for linkage disequilibrium in five markers (P=0.0001-0.03). The frequency of allele 237 in marker D12S326 was equal among mothers and fathers of affected offspring; however, we observed a maternal transmission of allele 237 in 90% of the cases compared to a paternal transmission rate of 45% (c^2=-10.74;P<0.00001). We found no evidence for a parent-of-origin effect for any of the 12q markers and AR or tIgE. Our findings suggest that asthma susceptibility is dependant upon both inheritance of a given 12q genotype and inheritance of that genotype from one's mother (not father).

Program Nr: 1345 from the 1999 ASHG Annual Meeting

Genome Scan for Linkage to Gilles de la Tourette Syndrome. *C.L. Barr[1], K.G. Wigg[1], R. Kurlan[2], A.J. Pakstis[3], K.K. Kidd[3], D. Pauls[4], L.-C. Tsui[5], P. Sandor[1]*. 1) Psychiatry, Toronto Hosp Western Div, Toronto, ON, Canada; 2) Neurology, University of Rochester School of Medicine, Rochester, NY; 3) Genetics, Yale University School of Medicine, New Haven, CT; 4) Child Study Center, Yale University School of Medicine, New Haven, CT; 5) Genetics, The Hospital for Sick Children, Toronto, ON, Canada.

Gilles de la Tourette Syndrome (TS) is a neuropsychiatric disorder characterized by both motor and vocal tics. Despite clear evidence for a genetic predisposition to TS from family, twin, and adoption studies, there have been no confirmed linkage findings. In this study we searched for linkage to TS in multi-generational families segregating TS using a panel of 386 markers with the largest interval between any 2 markers being 28 cM and an average distance between markers of 10 cM. We tested for linkage using an autosomal dominant model with reduced penetrance and using non-parametric methods. No significant evidence for linkage was found with parametric analysis. A Logarithm of the Odds (LOD) score of greater or equal to one under the autosomal dominant model was observed in twenty four of these markers in at least one of the families tested. No LOD scores greater than 2 were observed with any of the markers. For the non-parametric analysis, 8 markers were observed with a p value less than 0.00005 for significance evidence of linkage in at least one family. However caution should be used in the interpretation of the non-parametric analyses as this statistic (the Affected-Pedigree-Member method) is know to have a high false positive rate. Further support for linkage in these regions is required before linkage can be assumed.

Program Nr: 1346 from the 1999 ASHG Annual Meeting

Tardive Dyskinesia in Schizophrenia: Potential Role and Interaction of the Cytochrome P450 1A2 and Dopamine D3 Receptor Genes. *V.S. Basile[1], V. Ozdemir[2], M. Masellis[1], M.L. Walker[1], W. Kalow[2], H.Y. Meltzer[3], J.A. Lieberman[4], S.G. Potkin[5], F.M. Macciardi[1], J.L. Kennedy[1].* 1) Neurogenetics Section, Clarke Division, Centre for Addiction and Mental Health(CAMH), University of Toronto, Toronto, ONTARIO, Canada; 2) Department of Pharmacology, University of Toronto, Toronto, Canada; 3) Department of Psychiatry, Vanderbilt University, Nashville, USA; 4) Department of Psychiatry, University of North Carolina, Chapel Hill, USA; 5) Department of Psychiatry, University of California, Irvine, USA.

Long term neuroleptic treatment of schizophrenia induces tardive dyskinesia (TD) in predisposed patients. Upregulation of dopamine D2-like receptors may play a role in neuroleptic-induced TD. Following our initial report of DRD3 association with TD (Badri et al. 1996), research has currently focused attention on the D3 receptor (Steen et al., 1997; Basile et al., 1999; Segman et al., 1999). Inter-individual variation in neuroleptic metabolism may also contribute to an individual's propensity to develop TD. This study investigates genetic aspects of pharmacodynamic factors in TD by assessing the role of the D2, D3 and D4 receptor genes (DRD2,DRD3,DRD4), as well as pharmacokinetic factors by assessing polymorphisms of the cytochrome P450 1A2 and 2D6 genes(CYP1A2, CYP2D6). 112 neuroleptic treated schizophrenia patients were assessed for TD severity using the Abnormal Involuntary Movement Scale (AIMS). Analysis of covariance (ANCOVA), which incorporated risk factors for TD, was utilized to detect differences in TD severity across genotypes. The MscI polymorphism of DRD3 was found to be associated with TD ($F[2,95]=8.25$, $p<0.0005$) (Basile et al., 1999). Analysis of the Bsp120I RFLP of CYP1A2 revealed a significant association with TD ($F[2,99]=11.37$, $p<0.0005$). Assessing for a putative gene-gene interaction between CYP1A2 and DRD3, we have been able to detect a probable epistatic effect ($p<0.00007$). The remaining candidate genes did not exhibit any significant associations. Although replication is necessary, this study supports a role for both DRD3 and CYP1A2 in the pathogenesis of TD.

Program Nr: 1347 from the 1999 ASHG Annual Meeting

Follow-up of genomic screen for Autistic Disorder. *M.P. Bass[1], C.M. Wolpert[1], M.M. Menold[1], S.L. Donnelly[1], S.A. Ravan[2], T.D. Church[1], L. Zaeem[1], A. Zimmerman[3], J.R. Gilbert[1], H.H. Wright[2], R.K. Abramson[2], D.T. DeLong[4], J.M. Vance[1], M.L. Cuccaro[2], M.A. Pericak-Vance[1].* 1) Center for Human Genetics,Duke University Medical Center, Durham, NC; 2) W.S.Hall Psychiatric Institute, University of South Carolina, Columbia, SC; 3) John Hopkins University Medical Center, Baltimore, MD; 4) Division of Neurology, Pediatrics, Duke University Medical Center, Durham, NC.

Last year we reported results of our genomic screen for Autistic Disorder (AD), a neurodevelopmental disorder characterized by impairment in social, communicative, and behavioral functioning. Suggestive evidence for linkage (parametric or MLS LOD score ³ 1 or affected relative pair p-value < 0.05) was found for regions on chromosomes 2, 3, 6, 7, 15, 18, 19, and X. Chromosome 7, 15, 18, and 19 presented the most promising results and were prioritized for follow-up in an expanded data set of AD families (N=89). All affected individuals included in this study met DSM-IV/ICD10 criteria for AD, as determined by the Autism Diagnostic Interview-Revised (ADI-R) (Lord et al., 1994). We also genotyped a potential region on chromosome 13 (Vieland et al., 1998). The findings on chromosomes 13 and 18q were not replicated in these data, although the region on 18p did give slightly positive parametric results (maximum LOD score of 0.61). Chromosomes 15 and 19 continue to give evidence of linkage in our data set (maximum LOD score of 2.04 and maximum MLS of 1.21 for the regions on 15 and 19, respectively). Chromosome 7 data also remained interesting and is reported elsewhere (Ashley-Koch et al., this conference). In addition to the linkage findings, chromosomes 7 and 15 have associated cytogenetic abnormalities in the regions of interest. Updated results and follow-up analyses of additional promising regions will be presented.

Program Nr: 1348 from the 1999 ASHG Annual Meeting

Linkage analysis of five X-linked infantile spinal muscular atrophy (XL-SMA) families reveals a major disease locus located between Xp11.3-Xp11.2. *L.L. Baumbach[1], D. Dressman[2,3], A. Meindl[4], N.T. Bech-Hansen[5], E. Hoffman[2,3].* 1) Univ Miami Sch Medicine, Miami, FL; 2) Univ Pittsburgh Sch Med, Pittsburgh, PA; 3) Children's National Medical Center, Washington, D.C; 4) Ludwig Maximilians University, Munchen, Germany; 5) University of Calgary, Calgary, Alberta.

We have described an X-linked recessive 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 (*AJHG*, 61S, #1554, 1997). Other defining attributes include congenital onset of severe hypotonia, muscle biopsy indicative of neurogenic atrophy; and loss of anterior horn cells upon autopsy. Here we report clinical information and results of linkage studies conducted in six additional, unrelated XL-SMA families. Families were recruited based on the above diagnostic criteria, and four of the six new families evaluated for linkage to a candidate disease gene region, Xp11.3-q11.2, previously reported by our group in one family (*Hum Molec Genet.*, 4(7):1213-16, 1995). The remaining two families were not adequate for generation of a significant LOD score. Genomic DNA was analyzed using X-chromosome highly polymorphic markers. Concordance analysis was used to define maternal meiotic recombination breakpoints surrounding a disease gene region, followed by multipoint linkage analysis with additional DNA markers. Results were interpreted using the *LINKAGE* and *VITESSE* programs.

Results indicate that these four families are linked to the same candidate disease gene interval, Xp11.3-q11.2, also reported in the first family referenced above. Multipoint linkage analysis has been completed for three of these families and has refined this interval to a region defined by DXS991-DXS1003, with a maximum LOD score of 2.73. The total additive LOD score for these three families, and the family previously published, is 5.36 at a *Theta* of 0.00. There has been no evidence for genetic heterogeneity in these families. These results strongly support the existence of a major disease locus for XL-SMA between DXS 1003-DXS 991 (Xp11.3-Xp11.21).

Program Nr: 1349 from the 1999 ASHG Annual Meeting

Genome scan for quantitative traits involved in cardiovascular disease. *M. Beekman[1, 2], D.I. Boomsma[3], P. De Knijff[2], G.P. Vogler[4], C.C. Kluft[1], G.J.B. Van Ommen[2], R.R. Frants[2], P.E. Slagboom[1].* 1) Dept of Vascular and Connective Tissue Research, TNO-Prevention and Health, Leiden, The Netherlands; 2) Dept of Human Genetics Leiden University Medical Center, Leiden, The Netherlands; 3) Dept of Psychology, Free University of Amsterdam, Amsterdam, The Netherlands; 4) Center for Special Populations and Health, Pennsylvania State University, Pennsylvania, USA.

Genetic epidemiology has convincingly demonstrated the existence of genetic effects on quantitative traits such as cholesterol level, blood pressure and triglyceride level. The aim of our study is to map and identify quantitative trait loci (QTLs) for these risk factors of cardiovascular disease by performing a genome scan. In the past 16 months 91,000 genotypings were done for 85 loci at the most interesting chromosomes using two automated laser fluorescent DNA sequence analyzers (ALFexpress). From population based samples of Dutch, Swedish and Australian twins, which have been investigated in previous epidemiological studies, phenotypic data on apolipoprotein A1, A2, B and E levels, triglyceride levels, LDL and HDL cholesterol levels, blood pressure and BMI have been obtained. The 520 dizygotic (DZ) twin pairs in these populations are being genotyped for 229 highly polymorphic microsatellite markers with an average spacing of 18 cM. We composed for this genome scan 80 multiplex PCR reactions randomly typing loci in packages of 3 to 5 chromosomes, thereby enabling statistical analyses of chromosomes during the search. Our genotyping procedure requires for a complete 18 cM spaced genome scan less than 5 mg of genomic DNA isolated from blood or mouth swabs.

In the Dutch twin population (N=192 pairs) suggestive linkage (LOD of 2.8) was found at a 50 cM wide chromosomal region determining 50 percent of the variation in LDL cholesterol. This effect would account for most of the expected genetic contribution to LDL cholesterol. We are currently fine mapping this region in the Dutch twin sample by genotyping 232 parents and 50 additional siblings.

Program Nr: 1350 from the 1999 ASHG Annual Meeting

Confirmation of linkage of Van der Woude syndrome to chromosome 1q32: Evidence of association with STR alleles suggests possible unique origin of the disease mutation. *S. Beiraghi[1], A. Miller-Chrisholm[2], W.J. Kimberling[3], C. Sun[2], Y. Wang[2], L. Russell[2], M. Khoshnevisan[2], A.L. Storm[4], R.E. Long Jr.[5], P.D. Witt[4], M. Mazaheri[5], S.R. Diehl[2].*
1) Growth & Development, Univ Nebraska Medical Ctr, Omaha, NE; 2) Craniofacial Epidemiology & Genetics Branch, National Institute of Dental & Craniofacial Research, National Institutes of Health, Bethesda, MD; 3) Ctr for Hereditary Communication Disorder, Boys Town National Research Hospital, Omaha, NE; 4) Dept of Pediatrics, Univ of Arizona Health Sciences Ctr, Tuscon, AZ (A.L.S.); Pediatric Plastic Surgery, St. Louis Children's Hospital, St. Louis, MO (P.D.W.); 5) Lancaster Cleft Palate Clinic, Lancaster, PA.

Van der Woude (VWS) is an autosomal dominant craniofacial disorde with high penetrance and variable expression of its clinical features of cleft lip and/or cleft palate, lip pits and hypodontia. The gene has been mapped to a <2 cM region of chromsome 1q32. The aim of our study was to refine the localization of the VWS gene and to further assess possible heterogeneity. We recruited four multiplex VWS families. We used automated genotyping methods to characterize 19 short tandem repeat markers on chromosome 1 in the VWS candidate gene region. We performed two point and multipoint LOD score analyses using a high penetrance autosomal dominant model. All families show positive LOD scores without any recombination in the candidate region. The largest two point LOD score was 5.87. Our results are fully consistent with previous mapping of the VWS gene between D1S491 and D1S205. Our assay method for Short Tandem Repeat (STR) markers provided highly accurate size estimation of marker allele fragment sizes and, therefore, enabled us to determine the specific alleles segregating with the VWS gene in each of our four families. We observed a striking pattern of STR allele sharing at several closely linked loci among our four Caucasian VSW families recruited at three different locations in the U.S. This suggests the possibility of a unique origin for a mutation responsible for many or most cases of VWS.

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Program Nr: 1351 from the 1999 ASHG Annual Meeting

Mapping of a gene for non specific X-linked mental retardation : evidence for linkage to chromosomal region Xp21.3-Xp22.1(MRX54). *L. Ben Jemaa[1], V. desportes[2], R. Zemni[1], R. M'rad[1], F. Maazoul[1], C. Beljord[1], H. Chaabouni[1], J. Chelly[2]*. 1) genetique, faculte de medecine de tunis, tunis, tunisia; 2) INSERM U129 ICGM France.

X-linked nonspecific mental retardation (MRX) is a heterogeneous condition in which mental retardation (MR) appears to be the only consistent manifestation. Large genetic interval of assignment obtained on individuals families by linkage analysis, and genetic and phenotypic heterogeneity , are usually major obstacles to fine-map and identify the related disease genes. Here we report a large Tunisian family (MRX54) with an MRX condition. X-linked recessive inheritance is strongly suggested by the segregation of MR through 7 unaffected carrier females to 14 affected males in two generations. Two-point linkage analysis demonstrated significant linkage between the disorder and several markers in Xp21.3-22.1 (maximum LOD score Zmax =3.56, recombination fraction *=0 at DXS1202), that was confirmed by multipoint linkage analyses. Recombinant events observed with the flanking markers DXS989 and DXS1218 delineate a refined locus of about 2.7 cM in accordance with the physical distance between these two markers. The small interval of assignment observed in this family overlaps not only with 9 large MRX loci previously reported in Xp21.3-22.1, but also with two inherited microdeletions in Xp21.3-22.1 involved in non specific MR. Although the involvement of several genes located in Xp21.3-22.1 region cannot be ruled out, data reported in this study could be used as a starting point for the search of such gene(s).

Program Nr: 1352 from the 1999 ASHG Annual Meeting

The cathepsin D Ala224Val polymorphism is not associated with the risk of Alzheimer's disease. *T. Bhojak[1], S.T DeKosky[2], M.I Kamboh[1,2].* 1) Departments of Human Genetics; 2) Psychiatry, Univ Pittsburgh, Pittsburgh, PA.

Cathepsin D (Cat D) is an intracellular acid protease that is ubiquitously distributed in lysosomes. It is active in intracellular protein breakdown and has in vitro b-secretase-like properties. The Cat D gene is located on chromosome 11p15. A C®T (Ala®Val) transition at position 224 of the Cat D gene (exon 2) may be associated with increased pro Cat-D secretion and altered intracellular maturation. It has been hypothesized that increased activity of Cat D would result in altered APP processing and resultant increase in b-amyloid production. A recent study by Papasstiropoulos et al. (Neurosci. Lett. 1999; 262: 171-174) reported that carriers of the *CatD*T* allele were at a 2.4 fold increased risk for developing Alzheimer's Disease (AD) as compared to non-carriers, and this risk was independent of the APOE polymorphism. To confirm this association, we screened 446 sporadic, late-onset cases and 316 age-matched controls for the Cat D polymorphism. We found no significant association of the Cat D polymorphism with the risk of AD. The frequency of the *CatD*T* allele was 10% in AD cases and 9% in controls. Upon stratifying the data by *APOE*4* and non-*APOE*4* carriers, there was no significant difference in the frequency of the *CatD*T* allele between cases and controls (p=0.46) either. Our data indicate that the *CatD*T* Ala224Val polymorphism does not affect the risk of AD.However, a more detailed characterization of the Cat D gene may shed more light on its role in the development of AD.

Program Nr: 1353 from the 1999 ASHG Annual Meeting

A Genomic Survey for Substance Dependence in Families of Alcoholics. *L.J Bierut[1, 2], J. Rice[1,2], A. Goate[1,2], T. Foroud[2], H. Edenberg[2], C.R. Cloninger[1,2], H. Begleiter[2], M. Conneally[2], R. Crowe[2], V. Hesselbrock[2], T-K. Li[2], J. Nurnberger, Jr.[2], B. Porjesz[2], M.A. Schuckit[2], T. Reich[1, 2].* 1) Psychiatry, Washington University, St. Louis, MO; 2) Collaborative Study on the Genetics of Alcoholism.

Alcohol and substance dependence co-occur in the same individual more frequently than expected by chance alone. Evidence from family and twin studies support both common and specific addictive factors predisposing one to alcohol and other substance dependence. This is a report of a linkage study to locate genetic loci for marijuana and cocaine dependence in families of alcoholics. Subjects were part of the Collaborative Study on the Genetics of Alcoholism (COGA). Individuals who met criteria for both DSM-IIIR alcohol dependence and Feighner definite alcoholism were identified in substance abuse treatment settings, and their relatives were recruited as a sample at high risk for substance dependence. All subjects were interviewed using a semi-structured interview (SSAGA) that evaluated alcohol and substance dependence, and other psychiatric disorders. Male and female probands had high rates of marijuana and cocaine dependence (marijuana dependence 47% men 40% women; cocaine dependence 47% men 51% women), and there was significant familial aggregation of marijuana and cocaine dependence. A genomic screen for genetic loci linked to marijuana dependence (110 families - 159 affected sibling pairs) and cocaine dependence (89 families - 117 affected sibling pairs) was performed using affected sibpair analysis (ASPEX). The strongest evidence for linkage with marijuana and cocaine depende was at the same locus on chromosome 1 (Marijuana: Lod score=2.1 59% sharing; Cocaine: Lod score 1.93 60% sharing). This location on chromosome 1 has also shown evidence of linkage with alcohol dependence.

Program Nr: 1354 from the 1999 ASHG Annual Meeting

Primary Ciliary Dyskinesia: A genome-wide linkage analysis reveals extensive locus heterogeneity. *J.-L. Blouin[1], U. Radhakrishna[1], C. Gehrig[1], G. Duriaux Sail[1], A.J. Sainsbury[1], L. Bartoloni[1], M. Meeks[2], V. Dombi[1], D. Probst[1], B. Afzelius[3], M. Armengot[5], E. Chung[2], M. Jorissen[4], D.V. Schidlow[5], R.M. Gardiner[2], H. Walt[1], L. Van Maldergam[4], P.-A. Guerne[1], C.D. Delozier Blanchet[1], S.E. Antonarakis[1]*. 1) University and Cantonal Hospital of Geneva, and University Hospital of Zurich, Switzerland; 2) University College London, UK; 3) University of Stockholm, Sweden; 4) University of Leuven, and Institute of Pathology and Genetics of Loverval, Belgium; 5) University Hospital of Valencia, Spain and Allegheny University, PA.

Primary Ciliary Dyskinesia (PCD), is an autosomal recessive disorder affecting ciliary and flagellar movement (incidence: 1/30,000). Dysmotility to complete immobility of cilia results in upper respiratory tract infections, bronchiectasis and male sterility. 50% of cases have situs inversus (Kartagener syndrome). We have collected blood samples from 61 families. A genome-wide linkage search was performed in 31 multiplex families using 188 evenly-spaced microsatellites (19 cM interval). We performed a power calculation on our sample using q-value of 0.10 (scenario using a marker map density of 19 cM interval). The maxELOD was 18.22 (meanELOD=15.43). The sample was adequate to detect a Lod score of 3.74 at q=0.10 for a model in which 50% of the families were not linked to a locus. Linkage analysis was conducted on all families (all PCD), and in subgroups of families with situs inversus (PCD-SI), and in families with dynein arm deficiency (PCD-DAD). We analysed the genotype data with both parametric (PL, recessive model), and non-parametric (NPL) methods. No marker showed statistically significant values for linkage, but several chromosomal regions (4q, 5p, 8q, 16p, and 19q) were identified with suggestive evidence of linkage. The highest statistically suggestive scores were on chromosomes 4q (NPL p=0.00043, all PCD), 16p (NPL Zall=2.96, PCD-DAD), 8q (NPL Zall=2.85, PCD-DAD, PL ZH=2.43, PCD-SI), 19q (PL ZH=2.46, PCD-SI). At least 3 genes may be responsible for PCD supporting the genetic heterogeneity suggested by the spectrum of ultrastructural defects observed in cilia and flagella in PCD patients.

Program Nr: 1355 from the 1999 ASHG Annual Meeting

Fine mapping of region 1p32-p34.1 that contains the third major locus for autosomal dominant hypercholesterolemia. *C. Boileau[1], L. Villéger[1], J-P. Rabès[1], M. Devillers[1], M. Krempf[2], A. Cenarro[3], M. Farnier[2], M.J. Kotze[4], G.M. Kostner[5], M. Martinez[1], C. Junien[1], M. Varret[1]*. 1) INSERM U383 and U358, Paris, France; 2) CHU Hôtel Dieu, Nantes and Point Médical, Dijon, France; 3) Universidad de Zaragoza, Spain; 4) MRC Cape Heart Group, Tygerberg , South Africa; 5) University of Graz, Austria.

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 the LDLR and APOB genes account for ADH and we have shown that ADH is genetically more heterogeneous than conventionally accepted. We identified 13 Caucasian ADH families in which we excluded linkage to the LDLR and APOB genes thus demonstrating the implication of a new locus we named “FH3”. Genetic linkage was obtained in 4 pedigrees (HC2, S601, S150, S113) for chromosome 1 markers, localizing the FH3 locus in a 9 cM interval at 1p32-p34.1. By radiation hybrid mapping, four candidate genes (FABP3, SCP2, APOER2 and EPS15) at 1p32-p34.1 were located outside the critical region, demonstrating no identity with the FH3 gene. Heterogeneity tests estimated that 27% of these non-LDLR/non-APOB ADH families were linked to the FH3 locus. These results indicated the implication of a fourth locus called “FH4” that we are currently mapping by linkage analysis. To refine the localization of the FH3 gene, we have tested other regional polymorphic markers in the 4 originally linked pedigrees. In the HC2 family, the FH3 gene is now linked to a conserved haplotype defined by markers tel-D1S2892-D1S2722-D1S2645-cen that span an area of 2.8 cM. We are currently testing 5 new ADH families for which linkage was excluded to the LDLR and APOB genes. This should enable us to further shorten the genetic interval in which the FH3 gene is located. Furthermore, we are constructing a YAC contig of the area to undertake its physical mapping and map regional STSs.*tel-D1S2892-D1S2722-D1S2645-cen*.

Program Nr: 1356 from the 1999 ASHG Annual Meeting

Interference in the analysis of genetic marker data. *S. Browning, E.A. Thompson.* Statistics, University of Washington, Seattle, WA.

Analysis of identity by state (IBS) data at multiple linked markers requires assumptions about the crossover formation process. Haldane's Poisson process model is typically assumed. Other models, such as the chi-square renewal model can provide a much better fit to available data. We have developed methods for analyzing IBS data under a range of crossover models.

For a pair of individuals with IBS data at 433 markers (Puffenberger and Chakravarti, unpublished data),we compared the results of relationship inference under Haldane's model and under the chi-square model. A Markov chain Monte Carlo (MCMC) approach was used to calculate likelihoods under the chi-square model. For this pair, evidence of relationship increased slightly when the chi-square model (parameter m=2), rather than Haldane's model, was assumed (the LOD score for the most likely relationship vs. unrelated rose from 34.36 to 34.53). The most likely relationship for the pair was aunt-niece under both models.

Although the effect of the model on the relationship LOD score is small, it is important to know this. Also, interference can have a major effect on posterior identity by descent (IBD) probabilities, in one example changing them by 50%. A different MCMC approach allows estimation of these IBD probabilities among sets of individuals, at up to 12 linked loci, under alternative crossover models. We applied this method to data on trios of related individuals at 12 loci on chromosome 1 and on chromosome 19. IBD probabilities under Haldane and chi-square (m=2) models are compared.

We also investigated the amount of information in idealized IBD data (IBS data with infinitely dense and informative markers) to distinguish between models for crossing-over. Likelihoods on simulated data were calculated using a Monte Carlo method. We found that approximately 40 Morgans of IBD data from half-sibs are usually sufficient to distinguish between Haldane's model and the chi-square (m=2) model.

Work supported by NIH grant GM-46255 and a Burroughs Wellcome Fund PMMB training fellowship.

Program Nr: 1357 from the 1999 ASHG Annual Meeting

Linkage of familial schizophrenia to chromosome 13q32. *L.M. Brzustowicz[1,2], W.G. Honer[3], E.W.C Chow[4,5], D. Little[1], J. Hayter[1], M. Khan[1], L. Scutt[5], J. Hogan[5], D. Hayden[5], K. Hodgkinson[5], A.S. Bassett[4,5].* 1) Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ; 2) Department of Psychiatry, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, NJ; 3) Department of Psychiatry, University of British Columbia, Vancouver; 4) Department of Psychiatry, University of Toronto, Ontario; 5) Genetics Section, Schizophrenia Research Program, Centre for Addiction and Mental Health, Queen Street Division, Toronto, Ontario.

Over the past four years, a number of investigators have reported findings suggestive of linkage to schizophrenia with markers on chromosomes 13q32 and 8p21, with one recent study reporting significant linkage to these regions. As part of an ongoing genome scan, we evaluated microsatellite markers spanning chromosomes 8 and 13 for linkage to schizophrenia in 21 extended Canadian families (N=261 subjects). Markers were from the Cooperative Human Linkage Center linkage mapping screening set, with average spacing of 10 cM and average heterozygosity of 0.76. Families were analyzed using FASTLINK under both autosomal dominant and recessive models, using broad and narrow definitions of schizophrenia. All models produced positive lod scores with markers on 13q, with higher scores under the recessive models. The maximum three-point lod scores were obtained under the recessive broad model: 3.92 at q = 0.1 with D13S793 under homogeneity and 4.42 with a = 0.65 and q = 0 with D13S793 under heterogeneity. Positive lod scores were also obtained under all models for markers on 8p. While a maximum two-point lod score of 3.49 was obtained under the dominant narrow model with D8S136 at q = 0.1, multipoint analysis with closely flanking markers reduced the maximum lod score in this region to 2.13. These results provide independent significant evidence of linkage of a schizophrenia susceptibility locus to markers on 13q32, and are supportive of the presence of a second susceptibility locus on 8p21.

Program Nr: 1358 from the 1999 ASHG Annual Meeting

Cholestasis with lymphedema (Aagenaes syndrome): Genome screen and evaluation of candidate regions. *L. Bull[1], E. Roche[1], K. Eiklid[2], C. van der Hagen[2], A. Knisely[3], O. Aagenaes[2], N. Freimer[1]*. 1) UCSF, San Francisco, CA; 2) University of Oslo, Oslo, Norway; 3) University of Texas Medical Branch, Galveston, TX.

Cholestasis with lymphedema (CL), or Aagenaes syndrome, was first described in a Norwegian pedigree, in which the disease demonstrates probable autosomal recessive inheritance. Most Norwegian patients come from the same region, and are descended from a couple born circa 1570. CL is characterized by neonatal-onset cholestatic jaundice, accompanied by elevated levels of serum bile acids, bilirubin, and ALAT, and lasting 1-5 years. Recurrent cholestatic episodes occur in later childhood and adulthood. Lymphedema may be apparent at birth, or begin during childhood, and becomes chronic. Studies on urinary bile acids suggest no inborn bile acid metabolism abnormality. To identify the CL gene, we performed a genome screen using DNA from members of the Norwegian pedigree, and 385 autosomal microsatellite markers. A standard linkage analysis was not feasible because the structure of the pedigree is too complex, and too many samples are unavailable. Therefore, we designed a screening strategy to identify genome regions potentially shared identical by descent among several of these distantly related patients; two sib-pairs and a cousin pair were included. We identified candidate regions based on: 1) data consistent with linkage in the two sib pairs, 2) data consistent with linkage in the cousin pair, 3) evidence for marker haplotypes shared by affected individuals, and 4) evidence that particular marker alleles are more frequent than expected on the disease chromosomes. We paid particular attention to 5 regions containing genes previously found to be mutated in forms of hereditary liver disease or lymphedema (*BSEP*, *FLT4*, *PGY3*, *FIC1*, and *JAG1*). We have obtained no genetic evidence that CL is due to mutation in any of these candidate genes. We are currently evaluating 20 candidate regions identified in the genome screen on the basis of the genetic criteria outlined above. These regions are being evaluated by additional genotyping of the samples included in the genome screen, as well as in a larger sample of Norwegian CL patients.

Program Nr: 1359 from the 1999 ASHG Annual Meeting

Effects of order of analysis in mapping complex disease. *S.B. Bull, P. Nikolakakos, J. Biernacka.* Lunenfeld Research Institute and Public Health Sciences, University of Toronto, Toronto, ON, Canada.

In large genome scans with high-throughput genotyping, families are often typed in batches as they are recruited, leading to group-sequential-type data analysis. Evidence for linkage that emerges as the data accumulate may guide decisions to increase marker density in interesting regions or to set targets for additional family recruitment. Because of the practicalities of family studies, however, it is unlikely that the order in which families are typed is random and early results may differ systematically as well as randomly from final ones.

Using permutation sampling of affected-sib-pair families from a genome scan of complex disease, we investigated variability associated with using a subset of the families and with changing the order of analysis of accumulating data. With genotyping data for one chromosome, we examined the LOD score profile and the maximum value of the LOD and its location, based on the semi-parametric linkage model of Kong and Cox implemented in GHplus. We tabulated the frequency of detecting LODs above a specified threshold in the final analysis, given a maxLOD above/below a specified threshold in an early analysis. The effects of group sequential sample sizes, threshold values, and underlying genetic effects were explored in a large number of permutation samples. This approach is useful to quantify the extent to which early analyses may be potentially beneficial or misleading.

Program Nr: 1360 from the 1999 ASHG Annual Meeting

Selection Strategies for Disequilibrium Mapping of Quantitative Traits in Nuclear Families. *L.R. Cardon, W.O.C. Cookson, G.R. Abecasis.* Wellcome Trust Center for Human Genetics, University of Oxford, OX3 7BN, United Kingdom.

Quantitative traits provide effective descriptions of conditions as varied as allergy, obesity and reading ability. These phenotypes involve the interaction of a number of environmental and genetic factors, the identification of which is a major challenge for current mapping efforts. Allele sharing methods of linkage analysis allow the mapping of genetic factors to broad chromosomal resolutions. It is well established that the low power of these methods can be greatly improved by the use of selected samples, but the resolution of linkage analysis is limited by the number of recombination events in typical pedigrees.

Linkage disequilibrium extends for short distances across the genome and is a powerful fine-mapping tool. Family based methods of linkage disequilibrium mapping can distinguish association due to linkage disequilibrium from confounding effects due to population sub-structure. However, the power of these tests to detect effects that are in loose disequilibrium with the etiological mutation is low. It is likely that the efficiency of these methods will also improve if the most informative families are selected for analysis. We examined the performance of different sib-pair selection strategies, including single selection as well as concordant, discordant and extreme sib-pair strategies, and the effect of the marker and trait allele frequencies, the phase in which they are associated, and the amount of disequilibrium. Our results show that selection of extremely discordant sib-pairs is most powerful, although it is typically impractical. Selection of sib-pairs where at least one offspring has a very extreme phenotype can be an effective and practical alternative, and is more powerful than selecting extreme concordant sib-pairs. The different selection strategies favor different trait allele frequencies, and we show that this is an important consideration in marker choice.

Program Nr: 1361 from the 1999 ASHG Annual Meeting

Linkage analysis for a common multifactorial disease, the non syndromic familial orofacial cleft. *P. Carinci[1], L. Scapoli[2], M. Martinelli[2], F. Pezzetti[2], F. Carinci[3], S. Granini[2], I. Marchesini[2], M. Tognon[2].* 1) Dept Histology & Embryology, Univ Bologna, Bologna, Italy; 2) Dept Morfology & Embriology, Univ. Ferrara, Italy; 3) Chair of Maxillo-Facial Surgery, Univ Ferrara, Italy.

Nonsyndromic orofacial cleft is a common congenital anomaly, it represents an example of complex genetic trait in which clinical and genetic heterogeneity is observed. Although environmental influences on facial development have been described, a strong genetic component has been well established. The nature of the genetic contribution remains to be clarified, however an oligogenic mode of inheritance seems to be plausible. Several chromosome regions and candidate genes have been investigated for this malformation providing evidences of the involvement of at least three loci: OFC1 on 6p23, OFC2 on 2p13 and OFC3 on 19q13.2. In recent years, linkage analysis was greatly enhanced by the use of dense human genetic maps consisting of highly polymorphic microsatellite markers. At the same time, several statistic methods were developed to test the linkage hypothesis where complex diseases are concerned. We used this approach to investigate the OFC. For our OFC investigation, 45 families from Veneto region were enrolled. DNA was typed by PCR followed by electrophoretic analysis. Since the OFC malformation was found associated with chromosomal abnormalities involving the 6p23, this region is one of the most investigated for mapping study. We selected 8 microsatellite markers to test the presence of linkage in our family set. Locus heterogeneity for approx. 60% of families linked to D6S259 was observed1. A similar approach was applied for the 2p13 chromosome region study. Genetic heterogeneity was confirmed and evidence of linkage was obtained in previously identified 6p23-linked-families2. In a further investigation we studied the 19q13.2 region, by using the Lod score method. However both APM and TDT, model-free and non-parametric method respectively, support the hypothesis of an involvement of the BCL3 oncogene, which maps in 19q13.2, in causing the OFC3. 1Scapoli L. et al. 1997 Genomics 43:216-220. 2Pezzetti F, et al. 1998 Genomics 50:299-305. 3Martinelli M, et al. 1998 Genomics 51:177-181.

Program Nr: 1362 from the 1999 ASHG Annual Meeting

Genetics variation in two paraoxonase genes (PON1 and PON2) and risk of coronary heart disease in Hispanics and non-Hispanic Whites. *Q. Chen[1], J. Markey[1], R.F. Hamman[2], M.I. Kamboh[1]*. 1) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 2) Department of Preventive Medicine and Biometrics, University of Colorado, Denver, CO.

Serum paraoxonase (PON) is an HDL-associated enzyme which is believed to inhibit LDL oxidation and this may provide protection against the risk of coronary heart disease (CHD). Three PON-like genes, designated PON1, PON2 and PON3 have been identified and mapped to chromosome 7q21-q22. Two common polymorphisms in the PON1 (Gln192Arg) and PON2 (Ser311Cys) genes have been found to be associated with the risk of CHD in several Caucasian or Caucasian-derived populations. In this study we have examined the association of these two polymorphisms with the risk of CHD in two nested case-control cohorts of Hispanics (cases=82; controls=416) and non-Hispanic Whites (cases=93; controls=598). Among controls, the frequency of the Arg192 allele was significantly higher in Hispanics than non-Hispanic Whites (42.9% vs. 27.6%; $p<0.0001$), but the frequency of the Ser311 allele was comparable between the two groups (20.6% vs. 23.4%; $p=0.13$). In both groups, the genotype and allele frequencies of the two polymorphisms were not significantly different between cases and controls. Stratification of the PON1 data by PON2 Ser311/Ser311 genotype, however, showed a significant difference in the PON1/Arg192 allele frequency between cases and controls (29.2% vs. 41.7%; $p=0.015$) in Hispanics. Similarly, stratification of the PON2 data by PON1 Gln192/Gln192 carries showed a significantly higher frequency of the PON2/Cys311 allele in controls than cases (19.3% vs. 10.0%; $p=0.041$) in Hispanics, but not in non-Hispanic Whites. These inconsistent findings indicate that additional studies are needed to detect new mutations in the three PON gene cluster to identify functional mutations with regard to the risk of CHD.

Program Nr: 1363 from the 1999 ASHG Annual Meeting

A Single Nucleotide Polymorphism Map of a Bipolar Disorder Candidate Region on Chromosome 18q21.3-22.1.
Y.S. Chen, D.V. Leontiev, F.J. McMahon. Department of Psychiatry, University of Chicago, Chicago, IL.

A susceptibility gene for bipolar affective disorder may reside on chromosome 18q21-22. Based on multipoint linkage results using a dense set of microsatellite markers, we have selected a 24 cM region as a starting point for linkage disequilibrium mapping of this gene using single nucleotide polymorphisms (SNPs). In this region, we selected 114 sequence tagged sites (STSs), mostly gene-based, for SNP screening. Each STS is PCR amplified from a panel of 12 unrelated subjects (3 probands from 18q-linked families and 9 controls), the PCR product is purified, concentrated, and the forward strand is sequenced on an ABI 377 sequencer. Pherograms are then compared visually for candidate SNPs, based on the presence of 2 peaks at a single nucleotide position. To date, we have screened 15 STSs, representing ~22 kb of total sequence. We have detected 14 candidate SNPs and 2 small insertion/deletion polymorphisms (IDPs). Two SNPs were disconfirmed by reverse-strand sequencing, thus SNPs or IDPs occur at the rate of ~1/1500 bp in this region of the genome. The sequences from 3 STSs showed both homozygotes. About 1/3 of STSs contained more than one SNP, usually in complete linkage disequilibrium, but one STS contained 2 SNPs 36 bp apart that defined 4 different haplotypes. Automated resequencing of STSs is an efficient means for detecting sequence diversity for use in mapping complex traits.

Program Nr: 1364 from the 1999 ASHG Annual Meeting

Molecular study of X-chromosome mosaicism in Turner syndrome patients using the DNAs extracted from archived cytogenetic slides. *E.H. Cho[1], S.Y. Park[1], J.W. Kim[1], H.K. Ahn[2], J.Y. Jun[2], S.K. Choi[1]*. 1) Genetic Research Laboratory, Samsung Cheil Hospital, Seoul, Korea; 2) Obstetrics and Gynecology, Samsung Cheil Hospital, Seoul, Korea.

To study the X chromosome mosaicism in the cytogenetically pure 45,X Turner syndrome patients, we used the PCR technique with the DNAs extracted from archived cytogenetic slides. We amplified the DNAs using nested primers targeted to a highly polymorphic short tamdem repeat(STR) of the human androgen receptor gene (HUMARA) for the detection of X chromosome mosaicism. This assay is a very sensitive and useful method which can be applied to the DNAs extracted from archived cytogenetic slides to detect X mosaicism. We tested 50 normal Korean females to determine whether the HUMARA locus is highly polymorphic among the Korean. 85% of Korean population showed heterozygosity in the HUMARA locus. We analysed the 24 DNAs extracted from archived slides of patients and abortuses with Turner syndrome in cytogenetic analysis. We observed the heterozygosities of 50% from pure 45,X patients, 83% from patients with mosaic Tuner syndrome and 8.3% from abortuses of pure 45,X. Using the PCR technique of the HUMARA locus in the archived cytogenetic slides, we detected X chromosome mosaicism which could not be detected in cytogenetic analysis.

Program Nr: 1365 from the 1999 ASHG Annual Meeting

Systematic search for susceptibility genes in bipolar affective disorder - evidence for a disease locus at 10q26. *S. Cichon[1], G. Schmidt-Wolf[1,2], M. Pützstück[1], S. Hemmer[1], M. Albus[3], M. Borrmann[3], B. Lerer[4], E. Franzek[5], D. Lichtermann[5], B. Weigelt[5], C. Windemuth[2], T. Wienker[2], W. Maier[5], M. Rietschel[5], P. Propping[1], M.M. Nöthen[1]*. 1) Institute of Human Genetics, University of Bonn, Bonn, Germany; 2) Institute of Medical Statistics, University of Bonn; 3) Mental State Hospital Haar; 4) Hadassah University Hospital, Jerusalem, Israel; 5) Departments of Psychiatry, Universities of Würzburg, Bonn, and Dresden.

A potential susceptibility locus for bipolar affective disorder on chromosome 10q has recently been suggested. As part of a systematic genome scan for bipolar susceptibility genes, we genotyped 19 evenly spaced microsatellite markers covering whole chromosome 10 in a sample of 75 families (66 families from Germany, one family from Italy, 8 families from Israel) comprising 429 individuals. Parametric two-point linkage analysis was performed employing a dominant and a recessive genetic model. Two models of affection were used: affection status model (ASM)-I included only individuals with bipolar I (BPI) phenotype as affected, all other psychiatric diagnoses were coded as "unknown"; ASM II included all individuals with a diagnosis of BPI, bipolar II (BPII), schizoaffective disorder, bipolar type (SA/BP), and unipolar, recurrent (UPR). Using a dominant model and ASM II, D10S217 produced a LOD score of 2.86 at theta=0.05. With the neighbouring marker D10S587, a maximum LOD score of 1.48 at theta=0.15 was obtained. In a second step, we genotyped 14 additional markers spanning a 30 cM region containing D10S217 and D10S587. The best two-point LOD scores were found for D10S1757 (2.12 at theta=0.1), D10S216 (1.29 at theta=0.15), and D10S1727 (1.66 at theta=0.1). Non-parametric multipoint analysis using the GENEHUNTER program gave a maximum Z-all score of 3.2 (p=0.00098) at D10S216.

Program Nr: 1366 from the 1999 ASHG Annual Meeting

Meta-analysis of asthma and atopy. *A.R. Collins.* Dept Human Genetics, Univ Southampton, Southampton, England.

A central problem in complex inheritance is to map oligogenes for disease susceptibility prior to sequencing. Even large studies may have low power to map genes of small effect by linkage and association and confirmatory samples are always required. Combination of evidence, broadly called meta-analysis, offers a way forward. The simplest form of analysis uses published material and is useful for summarising evidence and delimiting candidate regions prior to follow-up studies. To apply meta-analysis to asthma and atopy 156 publications detailing linkage and association at 518 loci have been reviewed. Evidence from a marker may be summarised by a nominal significance level P and transformed to a corresponding lod (Z). Problems with published summaries are that P values may not have been corrected for multiple tests and many statistics have a minimum corresponding to Z=0. Nevertheless analysis of this material gives further insight into the importance and limits of the main candidate regions and their relationship to candidate genes. Important chromosomes include 6 with the HLA region being particularly significant. The HLA-DRB1 gene has 33 reports with atopy and also 5 reports with asthma for which the total lod is greater than 5. For chromosome 5 IL4 is associated primarily with atopy (Z=14) and ADRB2 with asthma (Z=9), suggesting different specificities. Other important chromosomes are 16,11,12,13 and 14. Retrospective collaboration, through the analysis of data already published individually offers the possibility of more rigorous analysis of these candidate regions. Such an analysis is being piloted for chromosome 5.

Program Nr: 1367 from the 1999 ASHG Annual Meeting

A quantitative trait locus influencing variation in ACTH precursor levels maps to chromosome 13 in Mexican Americans. *A.G. Comuzzie[1], A. White[2], L. Almasy[1], J.E. Hixson[1], J.W. MacCluer[1], J. Blangero[1]*. 1) Dept Genetics, Southwest Fdn Biomed Res, San Antonio, TX; 2) Schools of Biological Sciences and Medicine University of Manchester, Manchester, U.K.

Adrenocorticotropic hormone (ACTH) which stimulates the synthesis and secretion of adrenal steroids (e.g., cortisol, adrenal androgens, and mineralocorticoids)is synthesized as part of 2 precursors, POMC and pro-ACTH which are found in the circulation. In an attempt to identify genes involved in the regulation of this very important pro-hormone we have conducted a genome scan on serum levels of ACTH precursors in members of the San Antonio Family Heart Study. This analysis used 432 individuals distributed over 10 families, all of which were genotyped for 330 markers producing an average map density of 15cM. Linkage analysis was conducted using a variance component approach implemented in the program package SOLAR. Quantitative genetic analysis of ACTH precursors detected an additive genetic heritability of 51%. The maximum LOD score detected in the multipoint linkage scan was 2.62 ($p = 0.0003$) near *D13S788* on chromosome 13. Only one other region on chromosome 19 reached a suggestive level of significance (LOD = 2.0 between *D9S889* and *D9S254*). The 95% confidence interval for the chromosome 13 QTL overlaps the region containing the serotonin receptor 2A gene (*HTR2A*) which is a strong positional candidate gene for the regulation of POMC expression given the observed effects of serotonergic compounds on ACTH secretion in mammals. This work was supported by NIH grants HL45522, and MH59490.

Program Nr: 1368 from the 1999 ASHG Annual Meeting

A possible role for the interleukin-1 genes in early onset psoriatic arthritis. *A. Cox[1], N.J. Camp[2], M. Dale[1], F.S. di Giovine[1], L. Kay[3], D.J. Walker[3], G.W. Duff[1]*. 1) Div Molecular & Genet Medicine, Univ Sheffield, Sheffield, UK; 2) Genetic Research, IHC, Salt Lake City, Utah; 3) Freeman Hospital, Newcastle, UK.

Psoriatic arthritis is a complex, heterogeneous disorder with a genetic component to its etiology. The class I region of the major histocompatibility complex (MHC), in particular HLA-Cw6, is strongly associated with both psoriasis and psoriatic arthritis, but other genetic determinants have yet to be identified. Both the skin and joint manifestations of psoriatic arthritis are characterised by leukocyte infiltration and chronic inflammation. Therefore we have considered the genes of the interleukin-1 (IL-1) cluster as candidates, since IL-1 is one of the central mediators of these processes. The genes for IL-1 alpha, IL-1 beta, and the IL-1 receptor antagonist are clustered within 430kb on the long arm of chromosome 2. We investigated the relationship between IL-1 genotype and age at onset of psoriasis in psoriatic arthritis, since previous work had indicated that distinct IL-1 alleles may be associated with different age of onset groups in psoriasis itself. A cohort of 187 caucasian psoriatic arthritis patients and 840 healthy controls from the UK were genotyped for 4 biallelic markers in the IL-1 gene cluster. Two markers were associated with psoriatic arthritis in the overall dataset; IL-1B+3954 (p=0.004), and IL1RN+2018 (p=0.02). Comparison of the odds ratios of these two markers individually with the odds ratio for a composite genotype indicated that the IL1B+3954 was probably the primary association and that the IL1RN+2018 association was due to linkage disequilibrium. The other 2 markers investigated were not associated with the disease. The IL1B+3954 association was present only in the early onset cases, (age at onset <45 years), and stratification analysis indicated that it was primarily in the Cw6 negative patients (odds ratio (95%CI) = 1.9(1.2,3.0), n=103, vs. 1.2 (0.7,2.3), n=78). Thus, IL-1 may be implicated in early onset psoriatic arthritis, confirming previous results in caucasian psoriasis patients. A family-based replication study is in progress to confirm this result.

Program Nr: 1369 from the 1999 ASHG Annual Meeting

Genetic linkage of the Muckle-Wells syndrome to chromosome 1q44. *L. Cuisset[1], JPH. Drenth[2], JM. Berthelot[3], A. Meyrier[3], G. Vaudour[3], RA. Watts[4], DGI. Scott[4], A. Nichols[4], S. Pavek[5], C. Vasseur[1], JS. Beckman[5], M. Delpech[1], G. Grateau[1]*. 1) Genetique Moleculaire Humaine, EA2500, Inst. Cochin Genet Moleculaire et Service de Medecine Interne de l'Hotel-Dieu, Paris, France; 2) Department of Medicine, Division of General Internal Medicine, University Hospital St. Radboud, Nijmegen, The Netherlands; 3) Service de rhumatologie, L'Hotel-Dieu, Nantes,Service de nephrologie, Hopital Broussais, Paris, Service de pediatrie, Centre hospitalier general, Saint-Quentin, France; 4) Dept of Rheumatology, Ipswich Hospital, Dept of Rheumatology, West suffolk Hospital, United Kingdom; 5) Genethon, Evry, France.

The Muckle-Wells syndrome (MWS) is a hereditary inflammatory disorder characterized by acute febrile inflammatory episodes comprising abdominal pain, arthrisis and urticaria. Subsequent progressive nerve deafness develops and after several years the disease is complicated by multiorgan AA type amyloidosis (MIM 191900) with renal involvement and end-stage renal failure. The mode of inheritance is autosomal dominant, but some sporadic cases have also been described. No specific laboratory findings have been reported. The genetic basis of MWS is unknown. Using a genome wide search strategy in three families, we identified the locus responsible for MWS at chromosome 1q44. Our results indicate that the gene is located within a 13.9-cM region between the markers D1S2811 and D1S2882 with a maximum two-point LOD score of 4.66 (q= .00) at D1S2836 assuming full penetrance. Further identification of the specific gene responsible for MWS therefore provides the first biological element for characterizing MWS otherwise than on its variable clinical expression.

Program Nr: 1370 from the 1999 ASHG Annual Meeting

Linkage Analysis of Keloids. *K.D. Davis, S.M. Williams.* Microbiology, Meharry Medical College, Nasville, TN.

Keloids are benign, fibrous growths caused by abnormal wound healing that occur predominantly in darker pigmented races. The precise molecular and biochemical dysfunctions that lead to keloid formation remain unknown; however, there appears to be a strong genetic predisposition to keloid formation. We have undertaken a linkage analysis study to identify the chromosomal location of the predisposing gene(s) for the purpose of ultimately cloning and characterizing the genetic lesion. Markers linked to candidate loci were screened in two African-American families, of which nine out of twenty-three members are keloid formers. Based on the biochemical and molecular mechanisms involved in keloid formation, an array of candidates have been analyzed and excluded. These candidates include: alpha-1 type vii collagen, alpha-1 type II collagen, collagen x, alpha-1 collagen type xix, angiotensinogen, c-fos serum response factor, natural killer cell stimulatory factor, insulin-like growth factor, transforming growth factor beta, transforming growth factor beta 3, transforming growth factor beta type II receptor, epidermal growth factor receptor, interleukin 5 alpha receptor, interleukin-11 receptor alpha chain, interleukin 1 receptor antagonist, and the intracellular hyaluronic acid binding protein. A genome-wide search is currently underway. Approximately 620 cMs of 3490 cMs on the Genebridge 4 radiation hybrid map of autosomal chromosomes have been excluded. The genome-wide search will continue in an attempt to uncover a chromosomal location of the predisposing gene(s) responsible for keloid formation.

Program Nr: 1371 from the 1999 ASHG Annual Meeting

A score for MCMC genome screening. *E.W. Daw, E.A. Thompson, E.M. Wijsman.* Univ Washington, Seattle, WA.

Monte Carlo Markov chain (MCMC) techniques have shown promise in dissecting complex genetic traits. The methods introduced by Heath (AJHG 1997) and implemented in the program Loki have been able to localize genes for complex traits in both real and simulated data sets. Loki iteratively places quantitative trait loci (QTL) on chromosomes. The hit rate in each region estimates the posterior probability of linkage. We count hits not only by location, but also by QTL effect size, allowing estimation of the relative contribution of each QTL. Unfortunately, assessment of the significance of results has been difficult. Here, we introduce a score, the simulated log odds of placement (slop), for assessing oligogenic QTL detection and localization.

The slop compares hits on real chromosomes to hits on pseudo chromosomes unlinked to the trait. The pseudo chromosomes, generated by random gene drop, match the real ones for the map, allele frequencies, and missing data patterns in the observed pedigree structures. Both real and pseudo chromosomes are analyzed simultaneously with Loki. On a 2-dimensional grid of location and QTL effect:

slop = $\log_{10}$(max(0,(hits on real chr.-1))/(hits on pseudo chr.+1)).

The slop is similar to the lod score in being a log odds ratio, but differs in many other respects. For example, while lod scores are calculated under a single trait model, the slop is calculated with Monte Carlo integration over a large number of model parameters, including the number of trait loci and the relative additive/dominance effects at each locus. In analyses of real Alzheimer's Disease (AD) and simulated data, known genes produce slop scores over 3, but no pseudo chromosomes produce a slop this large. We use the real AD and simulated data to investigate the distributional properties of the slop in the presence and absence of trait genes, and to determine more precise significance thresholds.

Support: NIH grants GM 46255 and AG 05136.

Program Nr: 1372 from the 1999 ASHG Annual Meeting

Genome-wide scan for quantitative trait loci influencing HDL3 level in the Framingham Heart Study. *A.L. DeStefano[1,2], L.A. Cupples[2], M.D. Harmon[1], J.D. Keen[3], D. Housman[3], P.W.F. Wilson[1], R.H. Myers[1]*. 1) Boston University School of Medicine, Boston, MA; 2) Boston University School of Public Health, Boston, MA; 3) Massachusetts Institute of Technology, Cambridge, MA.

High density lipoprotein (HDL) cholesterol is recognized as a protective factor for coronary heart disease. Therefore, identification of genes that influence HDL level may be important in understanding the biochemical pathway of lipid metabolism, for assessing an individual's risk for CHD, and for targeting coronary prevention. HDL is composed of several subfractions, which may be under the control of separate genes. Examination of an individual subfraction may reduce the genetic complexity as well as the environmental noise present when considering total HDL level. In this study we focus on the identification of quantitative trait loci contributing to HDL3 level in the Framingham Cohort. HDL3 level was determined by double precipitation methods for Framingham Offspring blood samples obtained in 1987-91 and 1991-95. For genetic analysis, regression methods were used to adjust mean HDL3 level for physical activity index, alcohol and cigarette use, gender, age, estrogen use and menopausal status. A genome-wide scan (average distance 10cM) was conducted by the Mammalian Genotyping Service (Marshfield Medical Research Foundation) in 237 families (1167 genotyped individuals). Twopoint and multipoint LOD scores were computed using the variance component methods implemented in SOLAR for residual HDL3. Heritability was estimated to be .59. The maximum two-point LOD scores were 2.1 on chromosome 1, 2.0 on chromosome 7 and 1.9 on chromosome 10. Multipoint analysis resulted in a maximum observed LOD score of 1.9 on chromosome 7.

Program Nr: 1373 from the 1999 ASHG Annual Meeting

A genome scan for creatinine clearance among hypertensive siblings: The HyperGEN Network of the NHLBI Family Blood Pressure Program. *A.T. DeWan[1], J. Eckfeldt[1], D.C. Rao[2], S. Hunt[3], C.E. Lewis[4], S. Rich[5], D.K. Arnett[1].* 1) University of Minnesota, Minneapolis, MN; 2) Washington University, St. Louis, MO; 3) University of Utah Medical School, Salt Lake City, UT; 4) University of Alabama, Birmingham, AL; 5) Wake Forest University School of Medicine, Winston-Salem, NC.

Decreased renal function is often a complication of hypertension. Creatinine clearance measurements estimate the glomerular filtration rate, an indicator of renal function. While it is suggested that renal function has an underlying genetic component, there is limited information suggesting influential genetic regions or candidate genes contributing to the variability in creatinine clearance. As part of the HyperGEN study (a multicenter study to investigate the genetics of hypertension) we assessed creatinine clearance measurements in a large bi-racial sample of hypertensive sibs (n=171 African American (AA) and 270 white sibpairs). All participants were hypertensive prior to the age of 60, and the mean age of the siblings was 52 in AA and 60 in whites. To reduce the variability from non-genetic sources of variance in creatinine clearance, values were regressed on age, weight, pulse pressure, age at hypertension diagnosis, insulin replacement, educational status and physical activity in AA and age, lean body mass and pulse in whites. Standardized residuals were calculated separately for men and women in both racial groups. We conducted multipoint Haseman-Elston linkage analysis using MAPMAKER/SIBS with 387 anonymous markers (CHLC8 marker set). Our best evidence for linkage to creatinine clearance was on chromosome 3 at 166.3 cM (LOD = 2.38) and chromosome 15 at 38.3 cM (LOD = 2.03) in African-Americans and on chromosome 1 at 158.2 cM (LOD = 2.05) in Whites. There were also LOD scores greater than 1.0 on chromosomes 2, 7 and a second peak on chromosome 3 in African-Americans and on chromosomes 3, 6, and 10 in Whites. These data suggest there may be influential genetic regions contributing to renal function, and that these regions may differ by race.

Program Nr: 1374 from the 1999 ASHG Annual Meeting

Identifying marker genotyping errors and mutations in sibling-pair linkage data. *J.A. Douglas[1], M. Boehnke[1], K. Lange[2]*. 1) Dept Biostatistics, Univ Michigan, Ann Arbor, MI; 2) Dept Biomathematics, Univ California, Los Angeles, CA.

The widespread use of sibling-pair data for the investigation of complex diseases and quantitative traits, coupled with the availability of high-resolution genetic maps, demands appropriate methodology for the detection of marker genotyping errors and mutations. Previous methods have relied upon apparent Mendelian incompatibilities or close double recombinants in order to flag potential errors and mutations. Both, however, require genotype data from additional family members beyond the sibling pair to infer gene flow or to construct linkage phase. In many studies, especially those of late-onset complex traits where parental DNA is often unavailable, data restrict analyses to sibling pairs only.

Recently, we described a method for identifying siblings and markers with relatively unlikely genotypes based on a hidden Markov model in the context of multipoint mapping (Douglas et al. 1998). Assuming a map of tightly linked markers, we calculated the posterior probability of genotyping error at each marker for each sibling pair, conditional on all marker data for the pair and a prior error rate. We have now developed the FORTRAN 77 program SIB-MED to perform these analyses on a sample of sibling pairs. In addition, we have incorporated Monte Carlo simulation to assess the significance of each posterior error probability calculated from our sample. Genotypes characterized by unusually high posterior error probabilities may be considered for exclusion, review, or retyping.

We have examined the impact of genotyping errors and our method of error detection on both real and simulated linkage data. We illustrate that even moderate error rates of 1-2% can result in the loss of as much as 50% of linkage information, given efforts to fine map a putative disease locus. Though simulations suggest that our method generally detects only 20-50% of genotyping errors, depending on map density, marker position, and marker allele frequencies, it appears to flag exactly those errors that have the largest impact on linkage results. In many cases, removal of identified errors by our method restores most, if not all, of lost linkage information.

Program Nr: 1375 from the 1999 ASHG Annual Meeting

Multipoint linkage analysis of the pseudo-autosomal region using affected sibling pairs. *J. Dupuis[1], P. Van Eerdewegh[1,2].* 1) Genome Therapeutics Corporation, Waltham, MA; 2) Department of Psychiatry, Harvard Medical School, Boston, MA.

Methods for multipoint linkage analysis based on sibling amount of sharing identical-by-descent (IBD) are widely available, both for autosomal and X-linked markers. However, with this approach, data from the pseudo-autosomal region pose special challenges since same gender sibling pairs will share more material IBD in that region of the genome, with and without the presence of a disease-susceptibility gene. This increased sharing will be more pronounced for markers closely linked to the sex-specific region. Conversely, opposite-gender sibling pairs will share less material IBD. Failure to take this inequality in sharing into account may result in a false declaration of linkage if the study sample contains an excess of same-gender pairs due, for example, to gender specific prevalences. A linkage may also be missed when an excess of opposite-gender pairs is present. We propose a maximum LOD score method to take into account this expected increase/decrease in sharing when analyzing markers in the pseudo-autosomal region. We illustrate the new method with a simulation study on 500 sibling pairs using marker DXYS154 with published allele frequencies (10 alleles). Setting the probability of sampling a gender concordant pair at 68%, which would be achieved if one gender was 4 times more likely to be affected than the other gender, the uncorrected LOD score under H_0 (no linkage) ranges from 0 to 8, with 56 out of 100 replicates yielding LOD scores greater than 3. On the other hand, the corrected maximum LOD score did not produce any false positive results in 100 replicates. Even without gender distortion, the corrected LOD score improves power. The probability of exceeding a LOD score of 3 went from 28% to 55% in the case of a recurrence risk to offspring, l_O, of 1.5, and from 81% to 98% for l_O=2.0, by using the corrected LOD scores. For quantitative traits, we demonstrate with the Haseman and Elston method the same inflation in type-I error in the absence of an appropriate correction, and the inadequacy of permutation tests to estimate levels of significance when all phenotypic values are permuted irrespective of gender.

Program Nr: 1376 from the 1999 ASHG Annual Meeting

Screening of the SCA8 expansion. *A. Durr[1], G. Stevanin[1], A. Herman[1], A-S. Lebre[1], M. Frontali[2], A. Brice[1]*. 1) INSERM U289, Hopital de la Salpetriere, Paris, France; 2) Istituto di Medicina Sperimentale del CNR, Rome, Italy.

Inherited ataxias are widely represented among the group of neurodegenerative diseases caused by trinucleotide expansions. Recently, an untranscribed CTA/CTG repeat expansion in the spinocerebellar ataxia 8 (SCA8) gene was reported in patients with autosomal or recessive cerebellar ataxia but not in controls (Koob et al, 1999). We screened 352 chromosomes from French controls and 241 index patients of European origin, including 145 presenting with a non SCA1-7 autosomal dominant cerebellar ataxia (ADCA), 24 with recessive ataxia and 72 with sporadic ataxia. Repeat expansions above the threshold of 92 repeats (from 92 to 122) were found in 8 ADCA (5%) and 1 sporadic (1%) cases. The SCA8 expansion segregated in a small ADCA kindred but not in another. Alleles with 104 and 123 CTA/CTGs were also detected, however, in 2 healthy controls (1%), age over 57, with no known family history of neurological disorders. Two patients with Lafora's disease and essential tremor carried also expansions. Intermediate sized alleles (IA) ranging from 68 to 89 CTA/CTG repeats were detected in 2 patients with ADCA as well as in several patients from a single SCA3/MJD family. These IA did not segregate with the disease in the corresponding families and did not influence the phenotype of the SCA3/MJD patients. The expanded and intermediate sized alleles were moderately unstable during transmissions with changes ranging from -5 to +1 CTG repeats. In conclusion, the SCA8 expansion was found in ADCA and sporadic ataxic patients as well as in controls and patients with other neurological disorders from Europe. These results combined with the absence of cosegregation in a family with ADCA highly suggest that SCA8 expansions represent rare alleles that are not directly responsible for the disease.

Program Nr: 1377 from the 1999 ASHG Annual Meeting

Sampling strategies for the discovery of single nucleotide polymorphisms. *M.A. Eberle, L. Kruglyak.* Department of Molecular Medicine, Fred Hutchinson Cancer Research Center, Seattle, WA.

Discovery and mapping of single nucleotide polymorphisms (SNPs) is a major and rapidly growing enterprise in human genetics. A key issue when searching for new SNPs is what sampling strategies will be most effective in SNP discovery. In addition, it is important to understand how the frequency of SNPs restricted to a single sub-population (private) compares to those shared across multiple populations (public). We have formulated a model using the coalescent process that allows us to simulate various population histories in order to examine SNP frequencies within and across different populations. We will show results from models of populations with varying histories and degree of isolation and examine optimal sampling strategies for these models.

Program Nr: 1378 from the 1999 ASHG Annual Meeting

Genome-wide scan for schizophrenia in the Finnish population: Evidence for a locus on chromosome 7q22. *J. Ekelund[1,2], D. Lichtermann[1,2], I. Hovatta[1,2], P. Ellonen[1], J. Suvisaari[2], J.D. Terwilliger[3], H. Juvonen[2], T. Varilo[1], R. Arajärvi[2], M-L. Kokko-Sahin[2], J. Lönnqvist[2], L. Peltonen[1]*. 1) Dept. of Human Molecular Genetics and; 2) Dept. of Mental Health and Alcohol Research, National Public Health Institute, Helsinki, Finland; 3) Dept. of Psychiatry and Columbia Genome Center, Columbia Univ., NY, USA.

We have carried out a genome-wide scan in a schizophrenia study sample of 134 affected sib-pairs collected from the isolated population of Finland. We genotyped 370 polymorphic markers, followed by fine mapping of the two most promising regions, one on chromosome 1 and another on chromosome 7. The most significant finding was a two-point lod score of 3.18 with marker D7S486 using a dominant model and treating all individuals with any schizophrenia spectrum disorder as affected. Multipoint analysis with MAPMAKER/SIBS resulted in a MLS of 3.53 between markers D7S501 and D7S523 using the broadest diagnostic model, including MDD and Bipolar type I as affecteds in addition to the aforementioned phenotypes. Slightly higher lod scores for this region were obtained when only individuals from the late settlement region of Finland populated in the 16th century were included in the analysis. Some support was also obtained for linkage to chromosome 1q32.2-q41, a region previously identified in a genome-wide scan of a study sample from a regional sub-isolate of Finland. To increase the statistical power we also genotyped a study sample consisting of families with three or more schizophrenic children. In total 415 individuals in 89 families were analyzed. We obtained a maximal two-point lod score of 1.95 for marker D7S657, i.e. about 20 cM proximal to the maximal lod score in the sib-pair material. When pooling this data to that obtained in the analyses of the sib-pair material, the lod score increased for the more proximal markers while decreasing for the more distal markers. For the multipoint analysis, the marker order was confirmed by radiation hybrid mapping. No significant association was found for any of the markers, and therefore an even tighter marker map might be required to finemap the putative locus on chromosome 7.

Program Nr: 1379 from the 1999 ASHG Annual Meeting

Phenotypic variation at the chromosome 12 CFEOM1 locus. *E.C. Engle[1], B.A. Lee[1], C. Sener[2], B. Turgut[2], A.N. Akarsu[2], A.R. Khamis[3], A. Mousawi[3], E.I. Traboulsi[4]*. 1) Children's Hosp, Boston, MA; 2) Hacettepe University, Ankara, Turkey; 3) Al-Jazeirah Hospital, Abu-Dhabi, UAE; 4) Cleveland Clinic, Cleveland, OH.

The syndromes known as 'congenital fibroses of the extraocular muscles' (CFEOM) are ocular-motility disorders manifested as restrictive ophthalmoplegias with ptosis. Although these syndromes are thought to arise from dysfunction of the oculomotor nerve, their molecular etiology is unknown. Inherited forms of CFEOM have been described, and three genetic loci (CFEOM1-3) identified. CFEOM1, or 'classic' CFEOM, is AD with full penetrance, maps to 12cen, and affected individuals have bilateral infraduction and ptosis. CFEOM2 is AR, maps to 11q13, and affected individuals have bilateral exotropia and ptosis. CFEOM3 is AD, maps to 16qter, and is variably expressed -- some individual's eyes are down and out with ptosis, while others have only mild deficits in vertical gaze. Prior to this report, it was felt that each of these syndromes could be distinguished based on a family's clinical presentation alone.

We now report two different families that reveal phenotypic overlap between these syndromes. The first is an AD family with a partially penetrant and clinically distinct phenotype that most closely resembles CFEOM3, but maps to the CFEOM1 locus with a maximum lod score of 10.8 at D12S85. The second is an AR family not linked to the CFEOM2 locus, for whom a lod score of 2.0 (the maximum possible, given the family size and structure) is obtained at the CFEOM1 locus with reduction to homozygosity in the two affected children. Interestingly, this suggests that there are both AD and AR forms of CFEOM1.

These data establish that there is greater phenotypic heterogeneity at the CFEOM1 locus than previously reported, and blur our ability to distinguish the different CFEOM loci based solely on clinical presentation. Identifying the CFEOM1 gene mutations in these two families as well as in classic families should provide insight into the genetic mechanisms underlying CFEOM, and should be valuable to our understanding of the role these genes play in the normal and abnormal development of the oculomotor system.

Program Nr: 1380 from the 1999 ASHG Annual Meeting

Improved Identification of Most Probable Relationships in Relative Pairs. *M.P. Epstein, W.L. Duren, M. Boehnke.* Department of Biostatistics, University of Michigan, Ann Arbor.

Linkage analysis of a genetic trait can be compromised if the relationship of a pair of individuals is misclassified. Misclassification due to non-paternity, sample switches, or unknown adoption can lead to loss of power to detect linkage, either due to exclusion of families owing to apparent non-Mendelian inheritance, or to inclusion of pairs of individuals less closely related than assumed in the analysis. Sample duplications and misclassification of MZ twins as full sibs can lead to an increase in false-positive linkage results.

Boehnke and Cox (1997) introduced a likelihood ratio method to determine the most probable relationship in pairs of individuals. Multipoint probabilities of a pair's marker data are calculated assuming particular relationships given population marker allele frequencies and intermarker distances. Likelihood ratios comparing each potential relationship to the most likely relationship are calculated, and the strength of support for the most likely relationship determined. This method was implemented in a FORTRAN 77 program called RELPAIR. In data from simulated 20-cM genome scans of markers with four equifrequent alleles, this method correctly identified the true relationship with frequency 0.9994 for full sibs, 0.9980 for half sibs, and 0.9992 for unrelated pairs.

We have extended the work of Boehnke and Cox (1997) in several directions. The original method assumed autosomal marker data with no genotyping error, and only tested whether pairs of putative sibs were full sibs, half sibs, MZ twins, or unrelated. We now assess all possible pairs of individuals within a sample, include X-linked data in the multipoint probabilities, allow explicitly for the possibility of random genotyping error, and test for parent/offspring relationships. Extension to other relationships is planned. Using simulated data, we will present the classification rate estimates of true parent/offspring pairs and assess the impact of genotyping error and inclusion of X-linked data on relationship inference. The extensions will soon be included in the RELPAIR program available at www.sph.umich.edu/group/statgen.

Program Nr: 1381 from the 1999 ASHG Annual Meeting

MALDI-TOF mass spectrometry for high throughput SNP detection: Application to type 2 diabetes. *M.R. Erdos[1], A. Braun[2], K. Mohlke[1], K. Silander[1], S. Ghosh[1], F.S. Collins[1], for the FUSION (Finland-US Investigation of NIDDM) Study Group[1].* 1) NHGRI, NIH, Bethesda, MD; 2) Sequenom, Inc., San Diego, CA.

Matrix-Assisted Laser Desorption Ionization (MALDI) mass spectrometry has recently been introduced as a powerful analytical tool for the analysis of short DNA sequences by mass. Ions are generated by laser bombardment of DNA fragments embedded in a matrix of 3-hydroxypicolinic acid. These large DNA ions are accelerated through a path towards the detector measuring the time of flight (TOF) which is correlated with the mass of the DNA fragment. SNP analysis by MALDI-TOF is initiated by multiplex PCR amplification of genomic DNA, using primers flanking the SNPs of interest. A locus-specific primer which abuts the variable nucleotide of each SNP is then used in a primer oligo base extension reaction; primer extension is performed with DNA polymerase in the presence of dideoxynucleotides that terminate the reactions at a specific length determined by the allele present. The extended products are then purified and subjected to mass spectrometric analysis. In a pilot study, we have designed and successfully implemented the multiplex analysis by MALDI-TOF mass spectrometry of five genes with known polymorphisms that have been implicated in diabetes. The variants tested are SNPs in peroxisome proliferator-activated receptor gamma 2, insulin receptor substrate-1, insulin receptor substrate-2, uncoupling protein-2 and glucokinase genes. These genes are known to play a role in insulin sensitivity, insulin receptor signaling, obesity, and/or glucose metabolism, all traits which are associated with type 2 diabetes. Genotypes in the pilot study have been highly reproducible. We are currently extending this analysis to 582 probands of diabetic sib-pairs and 232 controls consisting of elderly non-diabetic Finnish subjects. Comparison of SNP allele frequencies between diabetic probands and controls should allow the identification or elimination of these candidate gene variants for association with type 2 diabetes in the Finnish population.

Program Nr: 1382 from the 1999 ASHG Annual Meeting

Screening of candidate genes for primary congenital lymphedema on 5q35.3. *A.L. Evans[1], G. Brice[1], V. Sotirova[4], P. Mortimer[2], J. Beninson[5], K. Burnand[3], J. Rosbotham[2], A. Child[1], M. Sarfarazi[4].* 1) Cardiological Sciences, St.George's Hospital , London, UK; 2) Department of Medicine, St. george's Hospital, London, UK; 3) Department of Academic Surgery, St. Thomas's Hospital, London, UK; 4) Surgical Research Center, Department of Surgery, University of Connecticut Health Center, Farmington, CT; 5) Department of Dermatology, Henry Ford Hospital, Detroit.

Primary lymphedema is a chronic tissue swelling, most frequently of the lower limbs, resulting from deficient lymphatic drainage. We have previously reported a locus for the primary congenital lymphedema (PCL), to the most telomeric portion of chromosome 5q35.3. In 5 PCL families, no recombination was observed with D5S408 (Z=10.03) and D5S2006 (Z=8.46) with a combined multipoint score of 16.55. This locus mapped below D5S2073 and WIAF-2213, but no telomeric flanking marker was identified. Recently, a second locus for lymphedema-distichiasis (lymphedema associated with a double row of eyelashes) has also been mapped to 16q24.3 (abstract submitted by Jeffery et. al.). Analysis of 20 pubertal onset lymphedema (Meige disease) families in our panel did not demonstrate any significant linkage to either of these two loci thus suggesting that at least one other locus remains to be discovered for this most common form of lymphedema. The vascular endothelial growth factor-C receptor gene (FLT4), an interesting candidate for PCL, is located within the critical interval. To date, we have found no disease-specific mutations. In addition, we have mapped five new candidate genes in to this vicinity using the GeneBridge4 radiation hybrid (RH) mapping panel and several other 5q35-specific RH clones. These genes have been completely sequenced in affected individuals but as yet, no mutations have been found. The screening of several other genes is currently in progress.

Program Nr: 1383 from the 1999 ASHG Annual Meeting

A novel craniofacial asymmetry mutation maps to mouse Chromosome 7 at the site of a random transgenic insertion. *E.T. Everett[1,2], J.K. Hartsfield,Jr.[1,3], E.M. Simpson[4].* 1) Dept Oral Facial Development, Indiana Univ Sch Dentistry, Indianapolis, IN; 2) Dept Dermatology, Indiana Univ Sch of Medicine, Indianapolis,IN; 3) Dept Medical & Molecular Genetics, Indiana Univ Sch of Medicine, Indianapolis,IN; 4) The Jackson Laboratory, Bar Harbor, ME.

A tendency toward sidedness is present in most normal human faces. Approximately 2-3 % of the general population and 34% of a dentofacial deformities population exhibit clinically apparent facial skeleton asymmetry. Facial asymmetry as part of a recognized syndrome (i.e., hemifacial microsomia) occurs in about 1 in 4000 livebirths and is second only to CL(P) as a birth defect involving craniofacial development. A recessive insertional mutation has been identified in the transgenic mouse line C57BL/6J-TgN(*Zfy1lacZ*)218BriEms that displays variable expression of anterior transverse malocclusion, craniofacial dysmorphia, and occasional maxillary bending. Homozygous transgenic mice also exhibit a variable but high incidence of runting and infertility. The absence of the phenotype in mice heterozygous for the transgene insertion suggests that this is due to a loss of function of an endogenous gene. Fluorescence in situ hybridization (FISH) analysis utilizing the transgene construct as a probe and hybridization to metaphase chromosomes prepared from a female homozygous transgenic mouse, maps the insertion of the transgene complex, and thus the mutation, to the distal region of mouse Chromosome 7, band F5. This region is syntenic with human chromosomes 10q, 11, and 16p. Immediate efforts are underway to clone DNA sequence flanking the integration site to facilitate the goal of identifying the endogenous gene(s) disrupted. The identification and characterization of genes that contribute to symmetrical growth and development of craniofacial structures will contribute to our understanding of the pathogenetic mechanisms leading to congenital and acquired disorders of facial skeleton asymmetry.

Program Nr: 1384 from the 1999 ASHG Annual Meeting

Association between angiotensin-converting enzyme and Alzheimer disease. *L.A. Farrer[1], G. Korovaitseva[2], E.A. Rogaeva[3], S. Petruk[2], S. Keryanov[2], S. Premkumar[1], Y. Song[3], T. Sherbatich[2], Y. Molyaka[2], N.D. Selezneva[2], S. Voskresenskaya[2], V. Galimbet[2], S. Sorbi[4], R. Duara[5], S. Gavrilova[2], P.H. St. George-Hyslop[3], E.I. Rogaev[2].* 1) Genetics Program, Boston Univ Sch Medicine, Boston, MA; 2) Russian Academy of Medical Sciences, Moscow, Russia; 3) Univ Toronto, Toronto, Ontario, Canada; 4) Univ Florence, Firenze, Italy; 5) Univ Miami Sch Medicine, Miami, FL.

The D allele for an insertion/deletion polymorphism in angiotensin-converting enzyme (ACE) has been linked to heart disease, stroke, and Alzheimer disease (AD). We investigated the ACE polymorphism in 151 patients meeting criteria for probable or definite AD and 206 ethnically matched controls from Russia and in 236 AD patients and 169 controls from North America using allele association methods and logistic regression. The D allele was more frequent among AD cases between ages 66 and 70 compared to age-matched controls in both the Russian (p=.02) and North American (p=.0006) data sets. In this age group, the effect of D (OR=11.2, 95% CI=2.9-44.0) appeared to be independent of and equal or greater in magnitude to the effect of APOE e4 (OR=7.8, 95% CI=3.5-7.4). ACE was not associated with AD in any other age stratum. Our results suggest that APOE and ACE genotypes may be independent risk factors for late-onset AD. In contrast to our findings, an association between the I/I genotype and AD was observed in 3 cohorts from the UK (Kehoe et al, Nat Genet 1999). However, the OR's were considerably less (1.8 to 2.7) and subjects were not stratified by age. These discrepent results suggest that an AD susceptibility allele is located elsewhere in ACE or a nearby gene. Alternatively, if D/D increases risk of stroke among persons below age 60, the D allele may influence AD risk by promoting small cerebrovascular infarcts, the cumulative effect of which is not manifest until about age 65. Evidence from our controls samples and a study of centenarians showing an increased frequency of D/D with age might explain the sudden diminution of the association after age 70. Thus, ACE may be an example of a gene with pleiotropic age-dependent effects on disease and survival.

Program Nr: 1385 from the 1999 ASHG Annual Meeting

Association between a CAG repeat polymorphism in the hKCa3 channel gene and spinocerebellar ataxia (SCA).

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Mutations in ion channels have been found to cause a variety of mendelian genetic diseases. HKCa3, a human calcium-activated potassium channel contains a polymoprhic polyglutamine repeat. Although not causative, longer polyQ tracts in this channel have been associated with psychiatric diseases in some studies. The spinocerebellar ataxias (SCAs) are a clinically and genetically heterogeneous group of neurodegeneative disorders. To date, nine different loci causing SCA have been identified: SCA1, SCA2, Machado-Joseph disease (MJD)/SCA3, SCA4, SCA5, SCA6 SCA7, SCA8 and dentatorubopallidolusian atrophy (DRPLA). Expansion of a CAG repeat in the disease genes has been found in five of these disorders. To test the involvement of this gene as a risk factor for ataxia we examined the genotypes of the hKCa3 CAG repeat polymorphisms in a case-control study in a population of 113 ataxic patients negative for the known SCA mutations and 152 age matched controls. Overall allele distribution between the three samples was similar with a CAG_{19} allele being the most common. One patient had an allele with 28 repeats which was 6 repeats longer than the next longest allele with CAG_{22}. However, since an allele of this size has been reported in one study as occurring in a normal individual (age unknown) it is not clear that this expansion is pathogenic. We next examined whether the CAG genotype at this locus was associated with the presence of ataxia. The overall genotype distribution was significantly different in patients with ataxia compared with normal controls by chi-square test (P=0.049). These results provide evidence for a possible association between longer alleles in the hSKCa3 gene and ataxia. These studies need to be replicated in a separate sample set and should examine this gene as a possible locus modifying age of onset in spinocerebellar ataxias.

Program Nr: 1386 from the 1999 ASHG Annual Meeting

A general approach to improving QTL detection in humans. *W.F. Forrest, E. Feingold.* Human Genetics, University of Pittsburgh, Pittsburgh, PA.

Most linkage studies for mapping quantitative trait loci (QTLs) in humans take one of two approaches. One approach is to use allele-sharing statistics based on identity-by-descent information with subjects ascertained because they possess anomalous phenotypes (e.g. discordant and/or concordant sibling pairs). The other approach studies the distribution of trait values given the inheritance vector (IBD information) for the pedigree. Both Haseman-Elston regression and variance components methods fall into this category. In this second type of study families need not be ascertained because of anomalous phenotypes, but typically they are at least to some extent. We show that these two approaches to QTL mapping contribute complementary rather than redundant information, and thus that statistics of the two types can be combined to form more powerful tests of linkage. We demonstrate this principle with a simple statistic for sibling pairs that combines the traditional Haseman-Elston statistic and the standard ``mean test'' for IBD sharing. We show with simulations that the combined statistic is more powerful than either test alone whenever the ascertainment is at least moderately based on phenotype. We also include suggestions for how statistics might be combined in more complicated situations than a sibling pair study.

Program Nr: 1387 from the 1999 ASHG Annual Meeting

A Transcript Map of the Chromosome 20 Type 2 Susceptibility Locus. *S.C. Fossey[1], J.A. Price[2], J.K. Pendleton[2], J.R. Snyder[2], C.S. Brewer[2], B.I. Freedman[3], S.S. Rich[4], D.W. Bowden[2].* 1) Dept Molecular Genetics, Wake Forest Univ Medical Ctr, Winston-Salem, NC; 2) Dept Biochemistry, Wake Forest Univ Medical Ctr, Winston-Salem, NC; 3) Dept of Internal Medicine, Wake Forest Univ Medical Ctr, Winston-Salem, NC; 4) Dept of Public Health Sciences, Wake Forest Univ Medical Ctr, Winston-Salem, NC.

Genetic linkage to chromosome 20q12-13.1 in Caucasian Type 2 diabetes families has been reported by multiple research groups (Bowden et al., Diabetes 46:882-886, 1997; Ji et al., Diabetes 46:876-881, 1997; Zouali et al., Hum Mol Genet 6:1401-1408, 1997; Ghosh et al., PNAS (USA) 96(5):2198-2206, 1999). HNF-4a, the gene responsible for maturity onset diabetes of the young, type 1, (MODY1) is localized to this same region, but mutations in HNF-4a do not account for the 20q linkage in Type 2 diabetes. An effort to positionally clone the diabetes gene(s) located on chromosome 20q12-13.1 is underway. To facilitate our efforts, we have constructed a physical map of this region, consisting of overlapping BAC and YAC clones (Price et al., AJHG, suppl:61, 1997). Concurrently, we have identified several regions of linkage disequilibrium within this interval (Price et. al., submitted). The genetic markers D20S888 and adenosine deaminase (ADA) show strongest evidence for linkage disequilibrium with Type 2 diabetes in this interval. We are generating a high resolution 2 Mb transcript map around each of these markers. Forty overlapping BAC clones around the ADA locus have been isolated and ordered. To this framework, 42 ESTs, 31 genetic markers and 11 known genes have been mapped to specific BACs. Flanking the D20S888 locus, we have ordered 21 BACs and localized 20 ESTs, 15 genetic markers and 6 known genes. We have surveyed 11 candidate genes, including ADA, HNF-4a, Syndecan-4, Receptor for Activated C Kinase 7 (Rack7), stress responsive serine/threonine kinase 2 (Krs-2), and Phospholipid Transfer Protein (PLTP1) for allelic variants associated with Type 2 diabetes. We are currently evaluating novel ESTs for contribution to Type 2 diabetes.

Program Nr: 1388 from the 1999 ASHG Annual Meeting

DFNA27, a new locus for autosomal dominant hearing impairment on chromosome 4. *R.A. Fridell[1], E.A. Boger[1], T. San Agustin[2], M.J. Brownstein[3], T.B. Friedman[1], R.J. Morell[1].* 1) Lab. Molecular Genetics, NIDCD, NIH, Rockville, MD; 2) NIDRR, DOEd, Washington D.C; 3) Lab. of Genetics, NIMH, NHGRI, NIH, Bethesda, MD.

Nonsyndromic hereditary hearing impairment is an extremely heterogeneous disorder. The continued identification of genes associated with hearing loss provides an effective means of uncovering essential components of the auditory system and promises an increased understanding of the molecular basis for sound transduction. We have ascertained a family segregating autosomal dominant nonsyndromic sensorineural hearing loss. In eleven affected individuals in this family, age of onset for the hearing loss was variable, ranging from pre-teens to late twenties. Affected individuals under the age of 40 years exhibit moderate to profound hearing loss (~30-90 dB). In older affected individuals, hearing loss is profound (> 90 dB). To map the locus responsible for hearing loss in this family, we first excluded known deafness loci and then performed a genome-wide scan with STR markers from the Weber 8 screening panel. Strong evidence for linkage was found to an approximately 15 cM region between markers D4S428 and D4S392 (maximum LOD= 4.76 at q = 0 for D4S3248). This region does not contain any other known nonsyndromic deafness genes and the new locus has been designated DFNA27. Progress in refining the DFNA27 genetic interval and identifying candidate genes will be reported.

Program Nr: 1389 from the 1999 ASHG Annual Meeting

Evidence that a dodecamer duplication in the gene HOPA in Xq13 is not associated with mental retardation. *M.J. Friez, J.W. Longshore, K. Sossey-Alaoui, R.L. Nelson, M.M. May, R.C. Michaelis, R.C. Stocco dos Santos, A.K. Srivastava, C.E. Schwartz, R.E. Stevenson.* Greenwood Genetic Center, Greenwood, SC 29646.

A recent study suggested that a dodecamer duplication in exon 42 of the HOPA gene in Xq13 may be a significant factor in the etiology of X-linked mental retardation. In an effort to investigate this possibility, we determined the incidence of the dodecamer duplication in two groups of non-Fragile X males and in two control groups (newborn male infants and male college students). The duplication was found in 4% of male patients from one non-Fragile X group, but in no male patients from either the other non-Fragile X group or families with X-linked mental retardation. In addition, the duplication was found in 1.5% of the newborn male and 2.5% of the male college student control populations. The incidence of the duplication was not significantly different between any of the groups in the study. These findings do not corroborate the findings of the previous study, and indicate that the HOPA dodecamer duplication does not convey an increased susceptibility to mental retardation.

Program Nr: 1390 from the 1999 ASHG Annual Meeting

A Genome Scan for Major Infectious Disease Susceptiblity Genes. *A.J. Frodsham[1], S. Best[1], K. Young[1], L. Zhang[1], M.R. Thursz[2], M. Chiaramonte[3], A.V.S. Hill[1].* 1) The Wellcome Trust Centre for Human Genetics, Oxford. OX3 7BN. UK; 2) Imperial College of Medicine, St Marys Hospital, London. W2 1NY. UK; 3) Instituto di Medicina Interna, Universita di Padua, 35126 Padova, Italy.

Changes in host genetics can powerfully affect a pathogen's ability to live in the hosts internal environment and under the surveillance of the host's immune system. In many infectious diseases it is apparent that many more people may be exposed to the pathogen than actually develop the disease. The reason for this may be environmental factors, differences in pathogen virulence or genetic differences between individuals. Evidence that host genetics play a role in susceptibility to infectious disease comes from twin and adoption studies, associations with HLA alleles and associations between candidate genes and infectious diseases. A Danish adoption study found that susceptibility to infectious disease was strongly influenced by heritable factors. The death of a biological parent before the age of 50 from an infectious cause was associated with a six-fold increased risk to the offspring of death from an infectious disease. Twin studies have shown a greater concordance in monozygous twins as compared to dizygous twins in a range of infectious diseases including pneumonia, leprosy, hepatitis B, malaria and tuberculosis. The aim of our study is to determine by linkage analysis the loci involved in the susceptibility to infectious disease. Using fluorescence based microsatellite technology and affected sibling pair linkage analysis, we have performed a genome wide scan using 32 extended pedigrees which contained a total of 61 independent, chronically infected sibling pairs. We used a panel of 299 polymorphic markers, which cover all 23 chromosomes with an average spacing of 10-20cM. Strongest evidence of linkage was found on chromosome 6, with a peak MLS score of 2.67 (p=0.0002) located at 6q26-27. We have analysed further microsatellites to fine map this region to an average spacing of 3cM and have localised the peak of linkage to a 1.2cM region on 6q26. We are investigating positional candidates in this region for evidence of association with infectious disease.

Program Nr: 1391 from the 1999 ASHG Annual Meeting

Genetic localization of the adult-onset autosomal dominant leukodystrophy (adld) gene to chromosome 5q. *Y. Fu[1], C. Coffeen[1], Y. He[1], L. Ptacek[1,2,3]*. 1) Human Genetics, Univ Utah, Salt Lake City, UT; 2) Department of Neurology, Univ Utah, Salt Lake City, UT; 3) Howard Hughs Medical Institute, Salt Lake City, UT.

The hereditary leukodystrophies represent a group of neurological disorders, in which complete or partial dysmyelination occurs in either the central nervous system (CNS) and/or the peripheral nervous system(PNS). Adult-onset autosomal dominant leukodystrophy (ADLD) is a slowly progressive, fatal multi-system neurological disorder, characterized by symmetrical wide-spread myelin loss in the CNS. The ADLD phenotpye is similar to that of chronic progressive multiple sclerosis(MS). We report the mapping of the gene that causes ADLD to an 6 cM region on chromosome 5q31. Linkage analysis of a previously reported American/Irish kindred yielded a LOD score of 5.72 at O=0.0 with the microsatellite marker D5S804, and linkage analysis of an additional family of Scottish/Irish origin increased the LOD score to 7.02 at 0=0.0 with D5S 804. Localization of the gene causing ADLD is the first step toward cloning the disease gene and characterizing function of the protein encoded by wild-type and mutant genes. New insight from this work will lead to better understanding of the pathophysiology of this disorder specifically and possibly to the molecular basis of leukodystrophy and MS in general. Ultimately such work will lead to better diagnosis and treatment for patients.

Program Nr: 1392 from the 1999 ASHG Annual Meeting

Monitoring myeloid and lymphoid chimerism with DNA markers is an useful tool in predicting early relapse after allogeneic BMT. *R. Galavotti[1], E. Trabetti[1], R. Fasolo[2], D. de Sabata[2], F. Benedetti[2], P.F. Pignatti[1].* 1) Section of Biology & Genetics, DMIBG, Univ. Verona, Italy; 2) Dpt. of Clinical Experimental Medicine, Univ. Verona, Italy.

Bone marrow transplantation is a powerful therapy used in a large number of hematological disorders. Relapse occurs in 10-30% of transplated patients, but in some cases, such as in CML, it can be easily reversed by a timely immunotherapy. It is therefore important to detect early relapse. From 1993 to 1998, 40 patients (CML, AML, ALL, MDS, MM, NHL), 18 males and 22 females, median age 36 (range 17-51 years) were transplanted in the BMT Unit of our Hospital. The median follow-up is 33 months (range 7-70). The conditioning regimen was TBI+Cytoxan for 36 and Busulphan+ Cytoxan for 4 patients. All the patients underwent frequent clinical and lab controls and peripheral blood (T and non-T) and bone marrow examination by DNA analysis at +21, +54, +90, +180 days, and then every year, after BMT. When available, cytogenetics and bcr/abl or other transcripts were used as additional data to follow the patients.DNA markers D1S80, ApoB, F8VWF, HUM-TH01, and others as necessary, were used. At present 26/40 pts are alive and well, with 100% donor myeloid and lymphoid cells. In 4 pts a transient mixed chimerism in the lymphoid lineage dissappeared within 90 days, and they have now a full donor engrafment. In 13 pts a mixed chimerism was demonstrated at any time during follow up. 1 pt with more than 5 year follow up has a mixed chimerism in lymphoid cells, that is compatible with a long survival. 8 pts (6 CML and 2 ALL) relapsed. In 6/6 CML pts DNA analysis detected the reapparance of autologous myeloid cells before any clinical evidence of disease. The data was confirmed in 5/6 cases by cytogenetics (Ph+) and bcr/abl transcript analysis. In 1 case there was no cytogenetic or transcript evidence, so that DNA was the only data predictive of relapse available. In conclusion, DNA analysis was a sensitive and general method to demonstrate donor engraftment in all patients studied. In many cases, as in MDS and MM, where no specific cytogenetic or molecular marker tests were available, it produced the only useful data.

Program Nr: 1393 from the 1999 ASHG Annual Meeting

Nonparametric disequilibrium mapping when haplotypes are available. *X. Gao, F.A. Wright.* Human Cancer Genetics, The Ohio State University, Columbus, OH.

A new method is described for the identification and mapping of haplotypes associated with disease in linkage disequilibrium studies. The method is applicable in situations where disease haplotypes and an appropriate sample of control haplotypes are available. By searching over observed haplotypes using variable window widths for contiguous markers, the haplotype most strikingly associated with disease is identified and mapped. The method naturally incorporates multi-allelic markers, and unlike other approaches is truly multipoint, in that multiple marker loci are used and the existing disequilibrium among markers (in both disease and control chromosomes) is built into the observed data. A statistic $S(a,b)$ is defined on the set of haplotypes beginning at marker position a and ending at position b, and is chosen to reflect striking associations of one or more of these haplotypes with disease status. The overall association evidence at marker position x is then $S^*(x) = max_{a£x£b}\, S(a,b)$. The approach is motivated by current progress toward large-scale association studies in outbred populations. However, the method appears to also offer considerable advantages for fine-scale mapping in isolated populations. One unique aspect of the method is the ability to distinguish and separately map multiple founding ancestral mutations. Data from cystic fibrosis and hereditary non-polyposis colon cancer are used as examples, and further refinements are proposed.

Program Nr: 1394 from the 1999 ASHG Annual Meeting

A family with X-linked epilepsy mapping to Xp11-Xq13. *C.C Garcia[1], M.R.H Buddles[1], M. Seager[2], J.A Goodship[1].* 1) Biochemistry and Genetics, Newcastle University, Newcastle-upon-Tyne , UK; 2) Northgate Hospital, Morpeth, UK.

We report a large X-linked epilepsy family. The clinical features are epilepsy with onset between teenage years and the third decade, learning difficulties and intermittent aggressive behaviour. The affected males are not dysmorphic. Some of the affected males have all three features whilst others have only epilepsy or learning difficulties with behavioural disturbance. Fragile X syndrome has been excluded.

17 microsatellite markers spanning the X chromosome were analysed in 17 family members including 8 affected males. GATA72E05(DXS7132) cosegregated with the disease. Recombinations with GATA69C12(DXS6810) mapping to Xp11 and GATA31D10(DXS6800) mapping to Xq13 defined a 30cM critical region. Monoamine oxidase A (MAOA) was a strong candidate gene given its chromosomal location and the mutation identified by Brunner (Science 262:578-580, 1993) in a family with a similar phenotype. However, the disorder in the family we describe does not segregate with a dinucleotide repeat polymorphism at the MAOA locus.

Program Nr: 1395 from the 1999 ASHG Annual Meeting

Heterozygote advantage of the MTHFR gene: Are heterozygous C677T/MTHFR women at lower risk of having children with nonsyndromic cleft lip with/without cleft palate? *D.A. Gaspar[1], R.C. Pavanello[1], M. André[2], S. Steman[3], M. Zatz[1], D.F. Wyszynski[4], S.R. Matiolli[1], M.R. Passos-Bueno[1]*. 1) Depto. de Biologia, Univ. de São Paulo, São Paulo, SP, Brazil; 2) Faculdade de Odontologia Universidade de São Paulo, São Paulo, Brazil; 3) Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil; 4) Laboratório de Epidemiologia Genética, Departamento de Salud Publica, Universidad de Buenos Aires, Argentina.

It has been shown in several populations that the C677T polymorphism of the 5, 10- methylenetetrahydrofolate reductase (MTHFR) gene is associated with an increased risk for neural tube defects (NTD), when both the mother and child have a TT genotype. Interestingly, Tolarova et al. (Am J Hum Genet 1998 63:A27) suggested that there is an increased proportion of TT homozygotes among CL/P Argentinean patients as compared to controls. These findings, however, are still controversial. We analysed the C677T polymorphism by comparing 77 Caucasian CL/P patients and 59 white mothers of CL/P patients to controls matched by age, socio-economic and ethnic background. The T allele and the TT genotype frequencies did not differ statistically among the tested groups and were in Hardy-Weinberg equilibrium both in controls and CL/P patients. However, unexpectedly, this polymorphic system was in Hardy-Weinberg disequilibrium among mothers of CL/P patients due to a relatively lower proportion of heterozygotes. Although this result could be a random variation, we hypothesised that CT heterozygotes might have an advantage towards the homozygotes in relation to this trait. The more independent confirmation of these results, showing a role of the maternal genotype predisposing to CL/P would provide new insights into the complex aetiology of this common birth defects. Supported by FAPESP, PRONEX, CNPq, HHMI.

Program Nr: 1396 from the 1999 ASHG Annual Meeting

Analytic Solution of Detection Rates when Genotype Errors Introduced into Family at Single Nucleotide Polymorphism Locus. *D. Gordon, J. Ott.* Lab Statistical Genetics, Rockefeller Univ, New York, NY.

Errors in human linkage data such as misreads by automated machines (allelic errors) or sample swaps (genotype errors) can increase type I error rates and reduce power in TDT tests. The goal of our analysis is to analytically determine the rate at which genotype errors are detected through Mendelian inconsistency in trios (parent 1, parent 2, child) that are genotyped at a single nucleotide polymorphism (SNP) locus that is in Hardy-Weinberg equilibrium (HWE).

In our error model, it is assumed that any of the three correct genotypes in a trio is replaced by a different genotype with constant probability, e. Further, it is assumed that errors occur randomly and independently. Hence, the probability of 1 to 3 errors occurring in a trio, conditional on at least one error occurring in the trio, is computed using the binomial distribution. It is also assumed that the trios arise from a population in HWE at the SNP locus. Using standard probability theory, the probability that errors are detected, or detection rate, for a randomly selected trio from the population may be calculated using the error rate e of genotype swaps and the allele frequency p of one of the two alleles at the SNP locus.

We compute this detection rate for various values of e and p . It can be shown that the theoretical maximum detection rate is 75%, but this detection rate is never achieved in practice. The values of e considered are 0.001, 0.005, 0.01, 0.02, 0.05, 0.1, and 0.2. The values of p considered are 0.01, 0.05, 0.1, 0.25, and 0.5. The maximum detection rate for these values is 66%, when e is 0.001 and p is 0.01. The minimum detection rate is 42%, when e is 0.001 and p is 0.5. For any fixed setting of e, the minimum detection rate occurs when allele frequencies are equal, i.e., $p = 0.5$. Also the detection rate appears to be much more sensitive to changes in the allele frequency p than to changes in the error rate e.

Program Nr: 1397 from the 1999 ASHG Annual Meeting

A common framework for model-based or model-free, twopoint or multipoint, linkage and/or linkage disequilibrium analysis of complex traits. *H.H.H. Goring[1], J. Ott[1,2], J.D. Terwilliger[1,3].* 1) Columbia U., NY; 2) Rockefeller U., NY; 3) NY State Psychiatric Inst., NY.

In linkage and/or linkage disequilibrium (LD) analysis, one computes the joint probability of a set of trait phenotypes (Ph) and a set of observed marker genotypes (G_M) in one's data. In conventional model-based analysis, this probability is computed as $P(Ph,G_M) = P(Ph|G_M)P(G_M) = S_{GD}\ P(Ph|G_D)P(G_D|G_M)P(G_M)$, by partitioning over all possible trait-locus genotype combinations for all individuals in the dataset (G_D). $P(Ph|G_D)$ is a function of the mode of inheritance, while $P(G_D|G_M)$ is a function of linkage and LD. By contrast, in model-free analysis methods, one computes this probability as $P(G_M,Ph) = P(G_M|Ph)P(Ph)$. One can estimate $P(G_M|Ph)$ without assuming anything about the genotype-phenotype relationships, if the ascertained samples have the same pedigree and phenotype structure (Ph) (e.g. affected sib-pairs, trios, or singletons). Though one does not model the genotype-phenotype relationships explicitly, the likelihood certainly depends on them, since $P(G_M|Ph) = S_{GD}\ P(G_M|G_D)P(G_D|Ph)$. $P(G_M|G_D)$ is a function of linkage and LD, and $P(G_D|Ph)$ is a function of how well the trait phenotypes predict the trait-locus genotypes. Since it is possible to find markers arbitrarily close to any trait locus, thereby increasing $P(G_M|G_D)$, the power of a study is dominated by $P(G_D|Ph)$, and the ascertainment and study design can thus be more important than the choice of statistical analysis method. We show how model-free analysis of linkage and/or LD can be performed by using deterministically assigned *pseudomarker* genotypes. In contrast to most conventional model-free methods, the pseudomarker analogs can be applied to different data structures jointly, thus using the total data more efficiently. These methods also have better statistical properties and are more powerful. We further show that twopoint analysis and multipoint analysis using *complex-valued recombination fractions* are algebraically isomorphic. This allows multipoint analysis, either model-based or model-free using pseudomarkers, to be performed with the same degree of robustness to trait-locus genotype errors as twopoint analysis.

Program Nr: 1398 from the 1999 ASHG Annual Meeting

Maximizing the Maximum Lod Score (MMLS): Application to Multipoint Analysis and Estimates of Gene Location. *D.A. Greenberg[1], P.C. Abreu[2]*. 1) Dept Psychiatry, Mount Sinai Medical Ctr, New York, NY; 2) Division of Biostatistics, School of Public Health, Columbia University, New York, NY.

The best available method for analyzing genetic linkage data remains an open question. We have demonstrated in previous work that performing a lod score analysis twice, once assuming dominant and once asuming recessive inheritance, and then taking the larger of the two values (designated MMLS) often has the most power to detect linkage. The power of the MMLS approach is comparable to analysis under the true model, even after correcting for multiple tests (Greenberg et al, 1998). We had demonstrated this using computer simulation under a variety of complex inheritance models. However, that previous work was done using two-point analysis, and the question has repeatedly arisen as to whether our findings will hold using multipoint analysis. In the current work, we extend our tests of the MMLS approach to multipoint linkage analysis and to a larger variety of generating inheritance models, including four heterogeneity models, four epistatic two-locus models, and two different additive models. We also examined the estimated position of the maximum in a chromosomal interval of 10 markers separated by recombination fractions of 0.1. We analyzed simulated data using lod scores (LS), lod scores assuming heterogeneity (HLOD), and NPL scores. Several conclusions can be draw:

First, our results indicate that, like two-point analysis, MMLS in multipoint analsyis provides good power to detect linkage even when the data originate from a variety of complex models.

Second, in all cases, we found that estimates of position were good to excellent for all three statistics,except that when heterogeneity was present, the lod score without heterogeneity performed poorly.

Third, the analyses using the lod scores and/or the HLOD had more power to detect linkage than NPL in all cases that we examined, although there was less difference between the methods for the additive models.

Program Nr: 1399 from the 1999 ASHG Annual Meeting

DFNA25, a novel locus for dominant, nonsyndromic, high frequency sensorineural hearing impairment. *C.C. Greene, P.M. McMillan, S.E. Barker, M.M. Lesperance.* Otolaryngology-Head & Neck Surgery, University of Michigan, Ann Arbor MI.

This is a study of a family of Czech descent from the midwestern United States with nonsyndromic dominant, progressive, high frequency sensorineural hearing impairment. Twenty-three of the patients studied are affected as compared to the 90th percentile for sex and age as measured in an unselected population (Robinson 1988). The youngest affected member was diagnosed at birth and the onset of hearing impairment for all affected members usually occurred in the first 2 decades of life. An estimate of the ability to detect linkage was calculated using the statistical software program SLINK (Ott, 1989). Ninety-nine-point-five percent of the 200 replicates of the pedigree generated by SLINK yielded a logarithm-of-odds (LOD) score over 3.0 (average maximum LOD score 9.75). Genotyping was performed using the polymerase chain reaction with radiolabeled primers and polyacrylamide gel electrophoresis. Linkage to known dominant loci for hereditary sensorineural hearing impairment was excluded by analyzing genotype data with the statistical software program MLINK (Ott, 1989). DNA from 50 patients (16 affected) informative for linkage analysis was then submitted to the Mammalian Genotyping Service for high-throughput genotyping across the entire genome. The genotype data for all markers was analyzed with MLINK. Linkage was detected to chromosome 12q21-q24 by analysis of a polymorphic marker within the phenylalanine hydroxylase (PAH) gene (LOD score of 4.02 at $q = 0.1$). Further genotyping of nearby markers and haplotype analysis revealed that the gene responsible for the hearing impairment in this family (DFNA25) maps to a 30 cm region defined by affected recombinants at D12S1052 and D12S1597. The region of human chromosome 12q21-24 has homology with a region of mouse chromosome 10. We are in the process of further narrowing the genetic interval by testing additional markers in the region. Haplotype analysis of these data and a discussion of candidate genes will be presented. (Supported by 1K08 DC 00161-01A1 and a grant from the Mammalian Genotyping Service of the National Heart, Lung and Blood Institute).

Program Nr: 1400 from the 1999 ASHG Annual Meeting

Haplotype Analysis of X Linked Migraine Pedigrees Refines the Migraine Susceptibility Locus to Xq24. *L.R. Griffiths[1], D.R. Nyholt[1], R.A. Lea[1], R.P. Curtain[1], P.J. Brimage[2].* 1) Genomics Res Ctr, School Health Science, Griffith Univ, Southport QLD, Australia 4217; 2) Institute of Neurological Sciences, Prince of Wales Hospital, Randwick, NSW 2031, Australia.

Recent studies in our laboratory have provided evidence for a X linked genetic component in familial typical migraine [1]. Using two large multigenerational pedigrees, designated MF7 and MF14, we were able to demonstrate significant excess allele sharing with a number of Xq microsatellite markers; MF7 giving a maximum NPL score of 2.57 at DXS1001, (P = 0.031) and MF14 giving a NPLmax of 2.74 at DXS1123 (P = 0.012). However, although combined analysis of the two pedigrees indicated excess allele sharing more towards the MF14 peak, the fact that the different peak regions of MF7 and MF14 were approximately 46cM apart, clearly indicated the need for finer gene mapping in these pedigrees. Therefore, to further localise and increase the information content across the implicated regions on chromosome Xq, the present study utilised additional microsatellite markers combined with haplotype analysis. Multipoint linkage analysis of genotype results was performed using the Sall scoring function in the GENEHUNTER-PLUS package, whilst reconstruction of the most likely haplotypes and determination of crossover events were calculated using the exact-likelihood method implemented in GENEHUNTER. Results from the study indicated significant evidence for X-linkage, with a peak LOD score of 2.388 (P = 0.0005) equidistant between DXS1001 and HPRT. Haplotype analysis was able to reduce this region further, indicating that the most likely localisation for the migraine susceptibility gene is the within a 5Mb region on the X chromosome. Hence we have provided further evidence for the location of a X chromosomal migraine gene and have been able to more firmly localise this locus to Xq24.

Program Nr: 1401 from the 1999 ASHG Annual Meeting

Spinal muscular atrophy with respiratory distress is heterogeneous and one form (SMARD) maps to 11q13-q21.

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Spinal muscular atrophy (SMA) is linked to markers on 5q13. SMA plus phenotypes are not linked to the SMA locus. Here we report an diaphragmatic SMA. In both SMA and diaphragmatic SMA severe limb muscle weakness is the common symptom. In contrast to SMA, where lumbar spinal cord, lower limbs and proximal muscles are predominantly affected, in diaphragmatic SMA cervical spinal cord, diaphragm, upper limbs and distal muscles are predominantly affected. Genome wide linkage scan in a consanguineous Lebanese family with five affected sibs (age of onset: 6-10 weeks) revealed linkage of SMARD to markers on 11q13-q21. For D11S1358 a maximum two-point LOD score of 3.16 at recombination fraction (Q)=0 was obtained. Multipoint linkage analysis yielded a maximum LOD score of 3.86 placing SMARD locus between D11S1883 and D11S917 which are 32.4 cM apart. Muscle histology revealed neurogenic atrophy without signs of reinnervation, and motor end plates presynaptic and postsynaptic degeneration. In one child the diameter of anterior spinal roots was reduced in specimen from the upper spinal cord. The locus on 11q13-q21 was supported by the haplotype analysis in a non-consanguineous German family with two affected children (onset: 9-12 weeks). In contrast, a third Italian family with two affected children (onset: birth) did not map to this region. It appeared that SMARD is autosomal recessively inherited. The histological findings offer two different pathophysiological concepts. Degeneration of anterior horn cells suggests dying forward atrophy. In contrast, end plate degeneration suggest dying back atrophy. In conclusion, diaphragmatic SMA is clinically and genetically heterogenous. One form (SMARD) maps to 11q13-q21.

Program Nr: 1402 from the 1999 ASHG Annual Meeting

Analysis of Chromosome 12 Candidate Genes in Late-onset Alzheimer Disease (AD). *J.M. Grubber[1], A.M. Saunders[1], L.H. Yamaoka[1], W.K. Scott[1], E.R. Martin[1], D.M. Hill[1], C.L. Standen[1], D.E. Schmechel[1], P.M. Conneally[2], G.W. Small[3], E.H. Lai[4], A.D. Roses[4], J.L. Haines[5], M.A. Pericak-Vance[1]*. 1) Dept.of Medicine, Duke Univ., Durham, NC; 2) Dept. of Medical and Molecular Genetics, Indiana Univ., Indianapolis, IN; 3) Dept. of Psychiatry, UCLA School of Medicine, Los Angeles, CA; 4) Genetics Directorate, GlaxoWellcome R&D, RTP, NC; 5) Program in Human Genetics, Vanderbilt Univ., Nashville, TN.

Three AD-causing genes: amyloid precursor protein, presenilin 1, and presenilin 2; and one susceptibility gene, apolipoprotein E, have been identified. Recent research indicates that the most promising location for a fifth AD gene is chromosome 12. We report results for 10 chromosome 12 candidate genes: neurotrophin-3 (NTF3); tumor necrosis factor receptor 1 (TNFR1); human complement component (C1r); oxidized low-density lipoprotein receptor (OLR1); islet amyloid polypeptide (IAPP); Kirsten rat sarcoma 2 viral oncogene (KRAS2); parathyroid hormone-like hormone (PTHLH); mitochondrial ATP synthase, beta subunit (ATP5B); human brain sodium channel 2 (hBNaC2); and interleukin-4 Stat (IL-4 Stat). These genes span approximately 65 cM from 12p13 to 12q13 and were chosen based on function, location, and availability of intragenic polymorphisms. The first 7 genes are located on 12p; the final 2 are located on 12q. ATP5B is in the 12p13-12qter region. The studies were performed on either: 1) 54-77 families collected by Duke, UCLA, Indiana University, and Massachusetts General Hospital; or 2) 348 families ascertained by Duke, UCLA, and NIMH. These two Caucasian, AD data sets contain at least 2 sampled affected individuals per family (³ 60) and some overlapping families. We performed sib-pair association studies (S-TDT) for all of the genes. In addition, we performed both parametric and non-parametric (MLS) two-point linkage analyses. The most significant p-value for the S-TDT was 0.10 for PTHLH. The maximum two-point lod scores for all of the data were 0.662 (parametric) for KRAS2 and 0.59 (non-parametric) for IAPP. We find no evidence that any of the ten candidate genes examined here is the sought after chromosome 12 AD susceptibility gene.

Program Nr: 1403 from the 1999 ASHG Annual Meeting

Genetic heterogeneity and epistasis in familial psoriasis: A meta-analysis of genome-wide studies. *C. Gu*[1], *A.M. Bowcock*[2]. 1) Division of Biostatistics; 2) Division of Human Genetics, Washington Univ Sch Medicine, St Louis, MO.

Psoriasis is a common inflammatory skin disease that affects ~2% of the Caucasian population. Evidence for a genetic etiology of psoriasis is revealed by numerous studies of its association with the human leukocyte antigens (HLA) complex, especially HLA-C and -B loci. Recently, additional susceptibility loci have been reported by researchers worldwide. These include loci within 17q25, 4q, 1q21-q23 and 20p. Although replication of these findings has not been always successfully achieved by independent groups, several high-quality genome-wide studies provided rich information for meta-analyses to quantitatively synthesize linkage evidences and to establish exploratory models to account for genetic epistasis and heterogeneity. We selected 8 such studies from an exhaustive MEDLINE search and performed various meta-analysis procedures. For example, a weighted inverse-normal method was used to derive a combined non-parametric p-value of 0.000372 for linkage to 17q, which gives ample evidence for PSORS2 and enhanced confirmation of the genetic heterogeneity of psoriasis. Further analysis using more sophisticated models is currently in progress, in which a novel test of heterogeneity is used by modeling study-specific covariates. These findings will aid in the understanding of the genetic etiology of psoriasis and in providing guidance for the design of future studies.

Program Nr: 1404 from the 1999 ASHG Annual Meeting

Fine linkage disequilibrium mapping of triple A syndrome in inbred North African families. *S. Hadj-Rabia[1], R. Salomon[1], A. Pelet[1], M.H. de Laet[2], B. Chaouchi[3], F. Bakiri[4], J.L. Chaussain[5], A. Munnich[1], S. Lyonnet[1]*. 1) Dept of genetics, Hopital Necker, Paris, France; 2) Dept of Surgery, Hopital d'Enfants, Brussel, Belgium; 3) Dept of Surgery, Hopital d'Enfants, Tunis, Tunisia; 4) Dept of Endocrinology, Hopital Bologhine, Alger, Algeria; 5) Dept of Endocrinology, Hopital St- Vincent-de-Paul, Paris, France.

Triple A syndrome (3A, MIM231550) is a rare autosomal recessive disorder characterized by ACTH resistant Adrenal insufficiency, Achalasia of the cardia, and Alacrimia. The 3A gene has been previously mapped to chromosome 12q13 in a 6 cM interval between loci D12S1629 and D12S312. In order to refine this localization, we have extended linkage analysis to 12 families (22 affected individuals) mostly originating from North Africa (7/12). We confirm that the 3A gene maps to the 12q13 region (Zmax=10.89 at Q=0 for D12S1604) and suggest that 3A is a genetically homogeneous disorder. Recombination events as well as homozygosity for polymorphic markers allowed to reduce the genetic interval to a 3.9 cM region between D12S368 and D12S312 loci. Moreover, total linkage disequilibrium was found at the D12S1604 locus between a rare allele and the mutant chromosomes in North-African patients (16/16). Conversely, only partial disequilibrium was found at either the D12S1651 (12/16) and the D12S1618 loci (6/16) that are telomeric and centromeric to D12S1604 respectively. Analysis of markers at 5 contiguous loci showed that most of the 3A chromosomes are derived from a single founder chromosome. As all markers are located in a 0 cM genetic interval and only allele 5 at the D12S1604 locus was conserved in all mutant chromosomes, we speculate that this mutation results from an ancient Arabian founder effect that occured before migration to North Africa. Since linkage disequilibrium at D12S1604 was also found in two patients from Southern Europe (France and Croatia), the founder effect might well extend to other Mediterranean countries. Taking advantage of a YAC contig encompassing the 3A minimal physical region, the gene was then mapped to a 1.7 Mb DNA fragment accessible to gene cloning.

Program Nr: 1405 from the 1999 ASHG Annual Meeting

A novel locus DFNA24 for congenital autosomal dominant hearing loss maps to 4q in a large Swiss German kindred. *F.M. Häfner[1], T. Linder[1], A.A. Salam[2], D. Balmer[3], A. Baumer[3], A. Schinzel[3], T. Spillmann[1], S.M. Leal[2].* 1) Deptartment of Otohinlaryngology and Head and Neck Surgery, University Hospital of Zurich, Zurich, Switzerland; 2) Laboratory of Statistical Genetics, The Rockefeller University, New York, NY; 3) Institute of Medical Genetics, University of Zurich, Zurich, Switzerland.

A novel locus for non-syndromic hearing loss has been identified in a Swiss German kindred with congenital bilateral autosomal dominant sensorineural hearing loss. This kindred has a history of hearing loss that dates back to the middle of the 19th Century. Audiometric and DNA samples were obtained on a total of 48 pedigree members. Twenty-six family members were considered to be affected and 22 unaffected. Affected family members' audiograms displayed a mild to profound hearing loss, mainly in the frequencies between 0.5 KHz and 4KHz. The affected members all had sloping audiograms affecting mainly the mid to high frequencies. Hearing impaired individuals with severe to profound hearing loss in the high frequencies also had moderate hearing loss in the mid and low frequencies. The hearing loss appears to be non-progressive with no correlation between severity of hearing loss and current age. No evidence for acquired risk factors predisposing to hearing loss was observed for any of the affected family members.

A genome scan was carried out on this family and a maximum multipoint lod score of 6.7 was obtained. The locus that segregates in this family maps to 4q. Additional work is being carried out to further refine the genetic region for this novel locus.

This work was supported by the NHLBI Mammalian genotyping service, NIH-NIDCD grant DC03594 and the American Hearing Research Foundation. .

Program Nr: 1406 from the 1999 ASHG Annual Meeting

Chromosome 16p13-cen and the MHC in multiple sclerosis. *J.L. Haines[1], M.E. Garcia[1], M.B. Gardiner[1], L.F. Barcellos[2], R.R. Lincoln[2], J.R. Oksenberg[2], S.L. Hauser[2], J.B. Rimmler[3], M.A. Pericak-Vance[3].* 1) Program in Human Genetics, Vanderbilt Univ Medical Ctr, Nashville, TN; 2) Dept. of Neurology, Univ. of California, San Francisco, CA; 3) Center for Human Genetics, Duke Univ., Durham, NC.

Multiple sclerosis (MS) is an autoimmune disorder of the central nervous system characterized by demyelination and inflammation and has a complex genetic architecture. Our previous genomic screen (MSGG, Nat Genet 13:469-71,1996) yielded 19 potential (lod > 1.00) chromosomal regions for harboring susceptibility alleles, including the MHC in 6p21 and regions 19q and 16p13-cen. We further estimated that approximately 1/3 of the total genetic effect in MS is explained by the HLA-DR2 association (MSGG, Hum Molec Genet, 7(8): 1229-1234, 1998). We also investigated a susceptibility locus on chr. 19q (Gardiner et al., ASHG abs#1674, 1998) and found evidence for an independent MS locus. We genotyped 5 markers in 16p13-cen, spanning 17 cM, in 98 multiplex families with 144 affected sibling pairs (ASPs). Fifty-two of these families were in the original screen. Analysis included two-point lod scores using dominant and recessive affecteds-only, low penetrance models and two-point ASP tests using ASPEX. We tested for interaction between the MHC and 16p13-cen with TWOLOC, a two-trait-locus ASP test. We found maximal two-point lod scores of 2.53, Q=0.15, for D16S2613 and of 1.70, Q=0.20, for D16S287. Two-point ASP analyses gave MLS scores of 1.22 and 1.85 for D16S2613 and D16S287, respectively. The 98 families were then stratified into three categories: 1) all affected individuals had the associated HLA-DR2 allele; 2) some affecteds had it; and 3) no affecteds had it. All strata had some evidence for linkage, with two-point lod scores of 1.00 or greater in this region, suggesting independence of the two loci. Independence of the chromosome 16 region was confirmed by TWOLOC analysis which gave an MLS of 7.27 in the general model and 6.28 in the additive model. HLA-DR alone gave an MLS of 4.22 and D16S287 alone gave an MLS of 2.72. These results suggest that 16p13-cen may harbor an independently-acting MS locus.

Program Nr: 1407 from the 1999 ASHG Annual Meeting

Allelic association and frequency of polymorphisms of the ADRB2 and CD14 genes in Icelanders with moderate to severe atopic asthma. *H. Hakonarson[1], U.S. Bjornsdottir[2], K. Kristjansson[1], J. Gulcher[1], E. Ostermann[1], C. Valiant[1], K. Carpenter[1], T. Gislason[2], D. Gislason[2], A. Gudnadottir[1], K. Stefansson[1]*. 1) deCode genetics INC., Reykjavik, Iceland; 2) Division of Allergy and Pulmonary Medicine, Vifilstadir University Hospital, Reykjavik, Iceland.

Several genes that map to chromosome 5q 31-33 reportedly influence the expression of the atopic asthmatic (AA) phenotype and specific polymorphisms (polym) in these genes may predispose to AA. In this regard, two common polym exist in the ADRB2 at amino acid 16 (gly for arg) and 27 (glu for gln) both of which are functionally relevant. The latter has been associated with BHR and elevated IgE levels in asthmatics. A polym in the CD14 gene (CD14/-159) was recently associated with the regulation of IgE levels. To analyze whether the allelic frequency of these polym is increased in Icelandic AA, we genotyped DNA from 94 AA age 12-40 years with moderate to severe asthma based on methacholine challenge tests, PFT values and response to drug therapy. DNA from 94 unrelated non-AA subjects was analyzed as control (CO). The population frequencies of the ADRB2 16 (A/G) and ADRB2 27 (C/G) alleles were not statistically different between CO (G16:55.2%; C27:62.5%) and AA (G16:55.8%; C27:60.5%). Moreover, the frequencies of the various allelic combinations of the ADRB2 16 and ADRB2 27 polym encoding for arg vs gly and gln vs glu (i.e., G16G27; A16C27; G16C27 and A16G27) were not statistically different between CO and AA. Similarly, the population frequencies of the C and T alleles of CD14 were not statistically different between the AA (C:58.5%) and CO (C:54.5%). Further, there were no statistical differences in the frequencies of the various allelic combinations of the CD14 and ADRB2 16 or ADRB2 27 polym. We conclude that: 1) The allelic frequency of the ADRB2 16, ADRB2 27 and CD14/-159 polym is not different between CO and AA in Iceland; 2) In contrast to previous reports, neither the ADRB2 16 or ADRB2 27 alleles (nor CD14/-159) were in linkage disequilibrium. These results suggest that polymorphisms in the ADRB2 and CD14 genes do not directly influence or predispose to the AA phenotype in Icelanders with moderate to severe AA.

Program Nr: 1408 from the 1999 ASHG Annual Meeting

A novel locus for Leber congential amaurosis (LCA) with anterior keratoconus mapping to chromosome 17p13.

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LCA is a clinically and genetically heterogeneous group of childhood retinal degenerations inherited in autosomal recessive manner. It is diagnosed at birth or during the first few months of life. Infants are totally blind or have greatly impaired vision, a fundal appearance ranging from normal to pigmented and an extinguished electroretinogram. LCA is caused by mutations in the RetGC-1 (17p13.1) RPE65 (1p31) and CRX (19q13.3) genes and a gene on chromosome 14q24. Anterior keratoconus is a bilateral non-inflammatory progressive corneal ectasia. Clinical signs include stromal thinning, conical protrusion, a ring-like deposition of iron around the base of the cone (Fleischers ring), fine vertical lines in the deep stroma and Descemets membrane (Vogts striae), anterior stromal scarring and enlarged corneal nerves. Keratoconus is most commonly an isolated disorder, although it has been described in association with many ocular and systemic disorders such as Downs Syndrome, mitral valve prolapse, and LCA. A two generation consanguineous Pakistani family with autosomal recessive LCA and keratoconus was identified. All affected individuals have bilateral keratoconus and congenital pigmentary retinopathy. Based on a whole genome linkage analysis we have mapped the first locus for this combined phenotype to chromosome 17p13. Linkage analysis gave a two point LOD score of 3.21 for marker D17S829. Surrounding this marker is a region of homozygosity of 23.2cM, between the markers D17S1866 and D17S960 within which the disease gene is predicted to lie. Mutation screening of the nearby Ret-GC1 gene, which has been shown to be associated with LCA, revealed no mutations in the affected individuals of this family. Furthermore Ret-GC1 was genetically excluded from the critical disease region based on the existing physical map. We therefore suggest that this combined phenotype maps to a new locus and is due to an as yet uncharacterised gene within the 17p13 chromosomal region.

Program Nr: 1409 from the 1999 ASHG Annual Meeting

An Autosomal Genomic Scan for Loci Linked to Indices of Insulin Sensitivity and Secretion in Nondiabetic Pima Indians. *R.L. Hanson, G. Imperatore, S. Kobes, W.C. Knowler*. DAES, NIDDK, Phoenix, AZ.

Type 2 diabetes mellitus is predicted by low insulin sensitivity and by diminished insulin secretion, and linkage analyses of these quantitative traits may identify genes important in the pathogenesis of diabetes. We conducted a linkage study of indices estimating insulin sensitivity and secretion among 521 nondiabetic adult Pima Indians from 212 sibships who had participated in an autosomal genomic scan (516 markers) for loci linked to type 2 diabetes.

The indices were calculated from fasting and two-hour serum insulin (I_0, I_2) and plasma glucose (G_0, G_2) concentrations obtained from an oral glucose tolerance test. The insulin sensitivity index [ISI = $(I_0G_0)^{-1}$] and the corrected insulin response [CIR = $I_2/(G_2(G_2\text{-}70\ mg/dl))$] were used as measures of insulin sensitivity and secretion respectively. Linkage was assessed by variance components methods.

The strongest evidence for linkage with ISI occurred on chromosome 14p (LOD=3.1), near D14S742; modest evidence for linkage was also found on chromosome 7q (LOD=1.2) near D7S479 in an area which is also linked to diabetes in Pimas (LOD=1.8, Am J Hum Genet. 1998; 63: 1130). The stongest evidence for linkage with CIR was found on chromosome 13q (LOD=2.2) near D13S800; additional evidence for linkage occurred on chromosome 1 (LOD=1.4) near D1S534, near another area which showed linkage to diabetes (LOD=2.5).

These analyses show strong evidence for a locus on chromosome 14 influencing insulin sensitivity and suggestive evidence for a locus on chromosome 13 influencing insulin secretion. They also suggest that the putative diabetes-susceptibility genes previously identified on chromosomes 1 and 7 may operate by influencing insulin secretion and sensitivity, respectively.

Program Nr: 1410 from the 1999 ASHG Annual Meeting

A correlated physical and genetic map of the refined PLS locus interval on chromosome 11q14. *T.C. Hart[1], S. Walker[1], S.A. Callison[1], P.L. Bobby[1], E. Firatli[2], D.W. Bowden[1].* 1) Wake Forest Univ Sch Medicine, Winston-Salem, NC; 2) University of Istanbul, Istanbul, Turkey.

Papillon Lefevre syndrome is an autosomal recessive condition characterized by palmoplantar hyperkeratosis and severe, early onset periodontitis that results in premature exfoliation of the primary and secondary dentitions. Three groups have independently localized a major locus for PLS to overlapping genetic intervals on chromosome 11q14. Only a few markers were common to the 3 different studies. To correlate teh 3 studies, we have constructed an integrated genetic map of the PLS critical region. We have extended our linkage studies of 16 consanguineous PLS families and sub-localized the PLS locus to the genetic interval flanked by D11S4147 and D11S931. Results of genetic analysis of the genetic markers spanning this interval suggest a genetic distance of approximately 2.8 cM. This corresponds to a physical distance of 4 Mb, containing at least 10 known genes and 40 ESTs. As part of our effort to identify the gene and specific mutation(s) that cause PLS, we have developed and correlated physical and genetic maps of the PLS candidate interval. We have developed a YAC contig that spans this genetic interval. We have confirmed the placement of 30 ESTs and genes on this contig. We have also evaluated expression of these ESTs/genes in gingiva as well as palmar and plantar skin. We present an integrated genetic map of the PLS candidate interval, correlated this map to the genetic map and determined which of these genes/ESTs are expressed in the tissues affected by the PLS phenotype. These findings should accelerate the search to identify the gene responsible for PLS.

Program Nr: 1411 from the 1999 ASHG Annual Meeting

Use of optical scanning capabilities to ensure accurate and efficient data entry in a multicenter genetic study. *C. Haynes[1], E. Crew[1], C. Blach[1], E.R. Hauser[1], J.E. Stajich[1], B.D. Slotterbeck[1], J.B. Rimmler[1], J.V. Vance[1], J.R. Gilbert[1], S.R. Brewster[2], A.D. Roses[2], P.K. Manasco[2], M.A. Pericak-Vance[1]*. 1) Duke Univ Med Ctr, Durham, NC; 2) GlaxoWellcome R & D, RTP, NC.

The identification of genetic risk factors in complex phenotypes is a rapidly expanding area. The success with which genes are discovered depends on many factors including the availability of large well-defined clinical populations. Multiple accrual sites allow genetic studies of complex phenotypes access to the large numbers of families needed for a successful study. However, capturing and maintaining the data necessary in such a global enterprise can be challenging. We have developed a prototype system that employs optical scanners to ensure consistent, dependable, and accurate data collection for a series of multicenter projects. Ascertainment is a two step process; first, there are established general forms used by all sites across several disease projects that collect general past medical history, ethnicity, and family history data common to all studies. The second phase is study specific and includes disease-specific clinical and epidemiologic forms. Data are collected, scanned, and verified at each accrual site which eliminates transcription errors and errors associated with manual data entry. Verified data are then transmitted electronically to the core database site (Duke University). Blood samples are collected using a specially designed form with peel-off bar coded labels that assure the identity of specimens. A key element is that all patient identifying information (name, addresses, contact information, etc) remains in a database local to the original accrual site; only anonymous information (patient identifier number, date of birth, clinical and family structure data, etc) are databased at the central core. This system insures patient confidentiality yet allows patient access for longitudinal studies. Each accrual site has specialized software which enables the site to track and view the complete set of data it has collected. Using this schema we have initiated global ascertainment networks in Parkinson disease, early-onset cardiovascular disease, osteoarthritis and asthma.

Program Nr: 1412 from the 1999 ASHG Annual Meeting

TDT with errors: a likelihood based approach. *S.C. Heath, J. Ott.* Lab Statistical Genetics, Rockefeller Univ, New York, NY.

The Transmission/Disequilibrium Test (TDT) is a non-parametric test for linkage. It does not requires large families, and can have more power to detect small effect loci than conventional linkage analysis methods, and has therefore been proposed as a method for performing genome scans for complex disease loci.

In the absence of genotype errors the TDT is an unbiased test for linkage. However, genotype errors lead to an inflation of the type I error rate whenever marker frequencies are unequal. We present a likelihood based approach for diallelic loci which tests whether the probability of transmission of a '1' allele from heterozygous parents to an affected child is different from 1/2. This is essentially the the same test as the TDT, but can allow for both genotype errors and missing genotype data. As well as the transmission probability (t), the likelihood depends on the genotype frequencies (p) and the error rate (e). These are often unknown, so the test uses their maximum likelihood estimates. To perform the test we calculate the likelihood ratio $T=2\ \mathrm{Log}(p',e',t')/\mathrm{Log}(p\bullet,e\bullet,t=1/2)$, which under the null hypothesis is approximately distributed as a chi-squared variable with 1 df. This test is unbiased in the presence of errors or missing (at random) genotype data. If, however, the data has been 'cleaned' before analysis (i.e., families with Mendelian inconsistencies have been removed), then e will be estimated at 0, and the estimate of t will (normally) be biased. It is therefore important that the test is performed on the original data. If this is not possible then an alternative approach, which we will also discuss, is to use both affected and unaffected offspring, estimating the transmission probability separately for each class of offspring and testing whether the two probabilities differ.

If TDT is to be a useful tool for detecting disease loci and given that genotype errors do exist, it will be necessary to use methods that either explicitly allow for errors or, at least, are robust to the presence of errors. It is hoped that the methods presented here will prove useful in this regard. This work was supported by NIH grants GM58757-01 (SCH) and HG00008 (JO).

Program Nr: 1413 from the 1999 ASHG Annual Meeting

Mapping of the Fanconi Anemia Complementation Group D (*FANCD*) gene to a 200 kb Region on Chromosome 3p25.3. *J.A. Hejna[1], C.D. Timmers[1], C. Reifsteck[1], D.A. Bruun[1], L.W. Lucas[1], P.M. Jakobs[1], S. Toth-Fejel[1], N. Unsworth[1], S.L. Clemens[1], D.K. Garcia[2], S.L. Naylor[2], M.J. Thayer[3], S.B. Olson[1], M. Grompe[1], R.E. Moses[1]*. 1) Dept. of Molecular & Medical Genetics, Oregon Health Sciences University, Portland, OR; 2) Department of Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, TX; 3) Vollum Institute, Oregon Health Sciences University, Portland, OR.

Fanconi anemia (FA) is a rare, autosomal recessive disease characterized by pancytopenia, sensitivity of cells to DNA cross-linking agents, and a predisposition to cancer. Of the eight known complementation groups, group D is rare, represented by only one family in the OHSU FA Cell Repository. Microcell-mediated chromosome transfer (MMCT) of a neomycin-marked human chromosome 3 into an immortalized FA group D fibroblast cell line, PD20, has localized the gene to chromosome 3p22-26. Marker analysis of a non-complemented microcell hybrid, PD20-3-8, sub-localized *FANCD* to an 8cM region centered around marker D3S1597, between D3S3591 and D3S3589. To narrow the region further, an additional 250 microcell hybrids were produced and analyzed. Of these, 7 remained sensitive to mitomycin C (MMC) and diepoxybutane (DEB). Three of these clones had lost the informative markers D3S3591, D3S1597, D3S1317, and D3S1263, suggesting that a large deletion had occurred during chromosome transfer. The remaining four clones retained the D3S1597 marker, suggesting the presence of small deletions adjacent to but not including D3S1597. A 1.2 Mb BAC/P1 contig was constructed, centered around D3S1597 and bounded by D3S3691, telomeric, and the gene ATP2B2, centromeric. Using BACs as probes, fluorescent in situ hybridization (FISH) analyses of the four additional non-complemented PD20 hybrids identified a small region of overlapping deletions. The overlapping deletions in these five independent non-complemented microcell hybrid cell lines localize *FANCD* to a 200 kb region on 3p25.3.

Program Nr: 1414 from the 1999 ASHG Annual Meeting

MISSENSE MUTATION E318G OF THE PRESENILIN-1 GENE IS ASSOCIATED WITH INCREASED RISK IN FINNISH ALZHEIMERS DISEASE PATIENTS. *S. Helisalmi[1, 2], M. Hiltunen[1, 2], A. Mannermaa[2], A.M. Koivisto[1], M. Lehtovirta[1], M. Ryynänen[3], I. Alafuzoff[1, 4], H. Soininen[1].* 1) Department of Neurology, Kuopio Univ Hospital and Kuopio Univ, Kuopio, Finland; 2) Chromosome and DNA laboratory, Kuopio Univ Hospital, Kuopio, Finland; 3) Unit of Clinical Genetics, Kuopio Univ Hospital, Kuopio, Finland; 4) Department of Pathology, Kuopio Univ, Finland.

Several different missense mutations of the presenilin-1 (PS-1) gene cosegregate with early onset Alzheimers disease (AD) in an autosomal dominant manner. The role of missense mutation E318G in exon 9 is controversial since it has been reported to cause AD as well as to be a rare polymorphism not related to AD. We have studied the frequency of the E318G variant in Eastern Finnish AD and control populations. Sequencing of the coding region of PS-1 from 16 unrelated familial cases revealed four patients with E318G mutation. No other PS-1 gene mutations or polymorphisms were detected. Additional 64 sporadic neuropathologically confirmed definite AD cases and 235 clinically verified controls and 35 neuropathologically confirmed controls were screened for the E318G variant using PCR-RFLP method. The E318G variant allele frequency was 25%, 11% and 4% in familial, sporadic and control cases, respectively. This data indicates increased risk of disease both in familial ($p = 0.005$; OR 7.6 (2.2-25.7)) and in sporadic AD cases ($p = 0.03$; OR 3.1 (1.1-8.2)) when compared to controls. In addition, genotyping of the microsatellite markers D14S77 and D14S1025, located at the vicinity of the PS-1 gene, showed that these markers were in linkage disequilibrium in the AD patients group carrying the E318G mutation. This data suggests that the E318G variant is likely nonpathogenic, because some controls also carried this change. However, it is possible that the variant is in linkage disequilibrium with pathogenic change elsewhere in PS-1 gene (e.g. promoter region).

Program Nr: 1415 from the 1999 ASHG Annual Meeting

Fine mapping and analysis of linkage disequilibrium in chromosome 15 linked autosomal recessive familial amyotrophic lateral sclerosis (*ALS5*). *A. Hentati[1], Y. Yang[1], K. Ouahchi[1], W-Y. Hung[1], F. Hentati[2], T. Siddique[1]*. 1) Dept Neurology, Northwestern Univ Med Sch, Chicago, IL; 2) Institut National de Neurology, Tunis, Tunisia.

Autosomal recessive amyotrophic lateral sclerosis (RFALS) is a rare form of motor neuron disease. RFALS is distinguished from the more common sporadic and autosomal dominant forms of amyotrophic lateral sclerosis by an earlier age at onset and slower progression of the disease symptoms. Clinical and genetic locus heterogeneity have been established in RFALS. We have previously mapped *ALS5*, the most common locus for RFALS to a 6-cM interval between D15S146 and D15S123 on chromosome 15q15-q22. None the markers that were available in this interval showed linkage disequilibrium with *ALS5* in three families from Tunisia. However, these markers were not evenly distributed and linkage disequilibrium was not excluded from two sub-segments between D15S214 and D15S514 and between D15S222 and D15S515. Here we report the refinement of *ALS5* to a smaller segment between D15S129 and D15S222. We have also isolated three new CA repeat markers from PACs that mapped to the ALS5 candidate region. One marker, 199I16R, maps between D15S214 and D15S514 and two markers, 112B14R and 18K11-2, map between D15S222 and D15S515. There was no linkage disequilibrium between 119I16R and ALS5. However, the alleles from markers 112B14 and 18K11-2 that are associated with the *ALS5* mutation were identical in two of the three families from Tunisia. These data strongly suggest *ALS5* maps in a 4-Mb segment between D15S214 and D15S222 and that more than one mutation causes *ALS5* in the Tunisian population.

Program Nr: 1416 from the 1999 ASHG Annual Meeting

New program: AUTOSCAN 1.0 automated use of linkage analysis programs. *T. Hiekkalinna, L. Peltonen.* University of California, Los Angeles, Department of Human Genetics, CA.

The use of linkage analysis programs consists of multiple file handling processes and production of input files specific for various statistical programs. This basically trivial process is time consuming and error prone since it repeats basic steps multiple times. Our aim was to develop a helper program to automate the tedious process of the creation of input files from genotype data of genome-wide scans, which would allow efficient analyses of several different phenotypic traits. In practice, this program enables the analyses of a whole genome-wide scan for all chromosomes in a single run. Our Unix-shell-script program AUTOSCAN automatically creates data files, uses MAKEPED (converts pre-linkage format files to linkage format), DOWNFREQ (estimates allele frequencies from pedigree data), and PEDCHECK (Mendelian checking) programs and finally starts statistical analyses via the ANALYZE package. ANALYZE performs parametric linkage analysis (MLINK), nonparametric affected sib-pair analysis (ASP), family-based association analysis (TDT, HRRR), and homogeneity testing (HOMOG). Input files for AUTOSCAN are the pedigree files for all chromosomes, the disease model file, the number of loci file, and the ANALYZE input file. The AUTOSCAN also allows easy handling of several phenotype traits and trait features by only changing the disease model file after each run. The program has now been tested by several research groups in the Department of Human Genetics, UCLA, and is available for downloading from our web page http://www.genetics.ucla.edu/software/autoscan.

Program Nr: 1417 from the 1999 ASHG Annual Meeting

Genome-wide linkage disequilibrium mapping of late onset Alzheimer's disease. *M.J. Hiltunen[1,2], A.J. Mannermaa[2], A.M. Koivisto[2], S. Helisalmi[1,2], M. Lehtovirta[2], M. Ryynänen[3], P. Riekkinen Sr[4], H. Soininen[2].* 1) Department of Neurology, Kuopio Univ Hospital, Kuopio, Finland; 2) Chromosome & DNA Laboratory, Kuopio Univ Hospital, Kuopio, Finland; 3) Unit of Clinical Genetics, Kuopio Univ Hospital, Kuopio, Finland; 4) A.I. Virtanen Institute, Kuopio Univ, Kuopio, Finland.

Alzheimers disease (AD) is a complex neurodegenerative disorder, for which several disease-associated loci have been located on various chromosomes. In order to find novel susceptibility genes for late onset AD, we have performed a population based genome-wide search using linkage disequilibrium mapping. To avoid population stratification, late onset AD patients and age-matched controls were carefully chosen from the same geographical area in Eastern Finland, where the population is mainly descended from a small group of original founders. Initial results from genome-wide search indicated regions in chromosomes 1, 3, 6, 8, 10, 13, 14, 18, 19, 22 and X to be in linkage disequilibrium in the AD population. The more precise examination of the chromosome 13q12 region indicated association with two adjacent microsatellite markers D13S292 and D13S787. Stratification of the AD patients and controls into the groups according to the apolipoprotein E, sex, and familial/sporadic status indicated that 13q12 locus was associated with female familial AD patients regardless of ApoE genotype. Based on the physical data from the region 13q12, markers D13S292 and D13S787 were estimated to reside in a 810 kb long YAC clone 754h7 together with two infant brain-derived ESTs and the H,K-ATPase a-subunit protein gene. The localization of these sequences at the linkage disequilibrium region suggests that they may be candidate genes involved in a sex-specific effect during development of AD.

Program Nr: 1418 from the 1999 ASHG Annual Meeting

Application of high-throughput SNP genotyping to complex endocrine diseases. *J.N. Hirschhorn[1,2], D. Altshuler[1,3], P. Sklar[1,3], S. Bolk[1], K. Lindblad[1], C. Lindgren[4], Y.-M. Lim[1], C.R. Lane[1], J. Nemesh[1], L. Groop[4], E.S. Lander[1]*. 1) Whitehead Institute/MIT, Cambridge, MA; 2) Children's Hospital, Boston, MA; 3) Massachusetts General Hospital, Boston, MA; 4) Lund University, Malmö, Sweden.

Common polygenic diseases have proven largely refractory to classical linkage analysis. It has been proposed that association studies using common sequence variants may provide a more powerful approach to understanding these clinically important genetic disorders. To help test this hypothesis, we developed rapid, reliable technologies to genotype single nucleotide polymorphisms (SNPs) and are applying these technologies to two common endocrine diseases.

Primer-directed, single base extension (SBE) in the presence of fluorescent ddNTPs reliably detects single nucleotide differences. By performing multiplex SBE reactions on multiplex PCR products, we can genotype 50-100 SNPs at once. Each SBE primer contains both a locus-specific sequence and one of 166 generic sequence tags. The multiplex SBE reaction is analyzed by hybridization to a generic array of 166 complementary oligonucleotide tags spotted on a glass slide; each SBE product hybridizes to a different location in the array. The generic array format and high degree of multiplexing allow fast, inexpensive SNP genotyping.

We are in the process of discovering coding SNPs (cSNPs) in over 300 genes relevant to endocrine disease. We have employed SNP-based methods to genotype these cSNPs in ongoing large-scale association studies for type 2 diabetes mellitus and abdominal obesity. The diabetes study involves 370 cases and parental controls (trios) and the abdominal obesity study involves 500 trios with extremes of waist-hip ratio. We perform transmission disequilibrium testing and thereby avoid false-positive associations due to ethnic admixture. In addition to novel cSNPs, we have typed all SNPs that have been previously associated with type 2 diabetes in case-control studies. Surprisingly few of the reported associations were found to be replicable, likely due to poopulation admixture confounding the original case-control studies and/or the rarity or absence of some SNPs in our populations.

Program Nr: 1419 from the 1999 ASHG Annual Meeting

A genome-wide scan for susceptibility genes for type 1 diabetes in scandinavian families.. *P. Holm[1], K. Åkesson[1], C.S. Bartsocas[3], G. Dahlquist[3], A. de Levia[3], C. Juliér[2], I. Kockum[1], M. Lathrop[2], H. Luthman[1], J. Nerup[3], F. Pociot[3], K.S. Rønningen[3]*. 1) Department of Molecular Medicine, Rolf Luft's Centre for Diabetes Research, Karolinska Institute, Sweden; 2) The Wellcome Trust Centre for Human Genetics, University of Oxford, UK; 3) European Consortium for IDDM Genome Scan (ECIGS).

Type 1 diabetes is a complex autoimmune disease, where particular alleles in the HLA region on chromosome 6p21 show a major contribution to the disease susceptibility. Previous studies of type 1 diabetes involving MZ twins and HLA identical sibs have shown that the major genetic contribution is from the HLA region. Other genes outside the HLA region are also involved. Identification of genes outside the HLA complex requires a large family and population material, preferably from homogenous populations. ECIGS (European Consortium for IDDM Genome Scan) was formed in order to perform a comprehensive linkage analysis with the aim to identify genes in other regions than HLA that may increase the risk for type 1 diabetes. A genome-wide scan was performed in 89 Norwegian families (102 concordant sib pairs), 183 Swedish families (220 concordant sib pairs), and 152 Danish families (174 concordant sib pairs), using 321 fluorescent markers with an average spacing of 11 cM. Non-parametric allele sharing methods were used for linkage analysis. Linkage was confirmed to two previously reported regions, IDDM1 on chromosome 6p21.3 (HLA) and IDDM15 located centromeric to HLA on chromosome 6, and two new regions also showed significant linkage to type 1 diabetes.

Program Nr: 1420 from the 1999 ASHG Annual Meeting

Methods for detecting genetic interactions using affected sib pairs. *P. Holmans, J. Rice.* Psychiatry, Washington Univ., St Louis, MO.

It is likely that several loci contribute to the genetic susceptibility to complex diseases, and that considerable interactions between these loci may exist. If two loci interact epistatically, a positive correlation is expected between the number of alleles shared identical by descent (ibd) at each locus, whereas heterogeneity should produce a negative correlation.

We consider three methods for detecting such interactions. Firstly, one can calculate the estimated ibd sharing for each sib pair at locations corresponding to lod score peaks obtained by single locus analysis, and test for a significant correlation. Secondly, one can select the pairs for which the estimated ibd is greater (if testing for epistasis) than a pre-determined value at the first locus. The test statistic for the second locus is calculated using only these pairs, and its significance determined by comparison with results obtained from samples of equivalent size randomly selected from the dataset. The third method models the probability that a sib pair inherit an allele ibd from a given parent as a logistic regression with the number of alleles shared ibd at the first locus included as a covariate.

Expected lod scores were calculated under a variety of two locus models, to determine the circumstances under which detection of interactions may be feasible. The statistical powers of the methods were compared by applying them to datasets containing sibships of various sizes simulated under these models.

Program Nr: 1421 from the 1999 ASHG Annual Meeting

Allele frequency estimation and comparison by SNP genotyping of genomic DNA pools. *B. Hoogendoorn, M.J. Owen, N. Williams, N. Norton, J. Austin, M.C. O'Donovan.* Dept Psychological Medicine, Univ Wales Col Medicine, Cardiff, Wales.

The ability to efficiently and cheaply genotype single nucleotide polymorphisms in large samples is fundamental to molecular genetic analysis of disease and, in particular, common complex diseases. We have combined primer extension analysis of SNPs with denaturing high performance liquid chromatography (DHPLC) (Hoogendoorn et al. 1999). After amplification of a fragment of DNA containing the SNP, an oligonucleotide primer is annealed immediately upstream from the polymorphism. In the presence of the appropriate dNTPs and ddNTPs, the primer is extended by one or more bases depending upon the sequence at the polymorphic site. The alleles are then distinguished on the basis of size of the extended product after DHPLC. We have used this method to screen pooled DNA samples to estimate and compare allele frequencies of SNPs. Our data suggest that this technique will allow accurate measurement of both the absolute allele frequencies in the pooled samples and the difference in allele frequency between pooled DNA samples from affecteds and controls. Thus, the analysis of pooled DNA samples by primer extension and DHPLC offers an efficient and relatively inexpensive method for screening large numbers of candidate polymorphisms in large sample sizes.

Program Nr: 1422 from the 1999 ASHG Annual Meeting

Power and false positive rates in two-stage genomic screens. *A.M Hossain, J.L. Haines, M.E. Garcia.* Program in Human Genetics, Vanderbilt Univ. Med. Center, Nashville, TN.

A 2-stage approach to genomic screens involves an initial screen using a low threshold and then a test of positive regions in a second data set. The goal is to reduce the amount of genotyping necessary and to eliminate false positive findings by requiring replication. To test the 2-stage screening strategy, we employed the Erehwon population of the GAW11 problem 2 simulated data sets (Greenberg, et al., Genetic Epi, in press) which consisted of 2500 families with sib-pairs and parents. The underlying genetic model had 3 loci, two of which interacted, spaced among 300 highly informative markers. We generated 1000 replicates of data sets of 100 or 200 families. We randomly picked 2 of the 1000 sets to create the initial and second data sets and this was done 1000 times. The data were analyzed using ASPEX. Peak regions were defined by choosing Maximized Lod Scores (MLS) of at least 0.5, 0.75, or 1.0 for any marker; markers with MLS lower than the criterion but located no more than 10cM away from the peak were considered part of the region.

Not surprisingly, sample size affected the power to replicate the true regions (using MLS=0.5, 58.1% power with 100 families and 81.6% with 200). However, replication of false positives remained high despite the increase in sample size (18.5% vs. 17.7%). Higher MLS critical values reduced the false positive rates but also decreased the power. With MLS=1.0, the false positive rates were reduced to 6% for both 100 and 200 families, but the power decreased to 31.4% (100 families) and 56.0% (200 families). Thus, a 2-stage approach using the same criterion in both data sets may not achieve its goal of weeding out false positive results while maintaining power.

Another problem in genomic screening is localization. The average distance between the true locus and the peak in either the first or second data set was 11-12cM, while the distance between peaks in the two data sets was 13-14cM. The distance between peaks for false replications was not substantially different at 12cM.

Program Nr: 1423 from the 1999 ASHG Annual Meeting

Transmission/disequilibrium test (TDT) approach at the van der Woude syndrome (vWS) locus in familial nonsyndromic cleft lip with or without cleft palate (NSCL/P). *C. Houdayer[1], V. Soupre[2], G. Morgant[1], A. Munnich[3], M.-P. Vazquez[2], M. Bahuau[1,2]*. 1) Service de Biochimie et Biologie Moléculaire, Hôpital Trousseau, Paris, France (biochimie.trousseau@trs.ap-hop-paris.fr); 2) Service de Chirurgie Maxillofaciale et Réparatrice et Stomatologie, Hôpital Trouseau, Paris, France; 3) Service de Génétique Médicale, Hôpital Necker Enfants-Malades, Paris, France.

NSCL/P is a most common congenital malformation condition in humans. Inheritance is generally regarded as multigenic, allelic variation at different loci (TGFa, TGFb3, RARA, etc.) determining a fraction of the genetic risk, as demonstrated by association/TDT studies. On the other hand, vWS is a rare autosomal dominant clefting condition with cardinal features of lower-lip pits (the highest penetrant trait), and CL/P or cleft palate (alone), present in half of the gene carriers. The *vWS* gene has been localized to a 1.6-cM region in 1q32, also containing highly polymorphic marker D1S3753 and flanked by D1S205 and D1S491. Some vWS families harbor a more or less extended deletion at this locus, indicating that vWS proceeds by haploinsufficiency. In order to investigate possible involvement of the *vWS* locus in genesis of NSCL/P, we opted for a TDT approach based on 45 NSCL/P trios selected from 45 multiplex NSCL/P families. Patients and their parents were screened using markers D1S205, D1S491, and D1S3753 (kindly provided by Jeffrey C. Murray, University of Iowa). As expected, no deletion was observed in any of the 45 kindreds. Chi square analysis for transmitted vs. non-transmitted alleles of the three markers yielded no value significantly departing from random assortment. These results indicate that either *vWS* is not a locus for NSCL/P or that a substantially larger cohort is needed for detecting association.

Program Nr: 1424 from the 1999 ASHG Annual Meeting

Fine-mapping of a putative schizophrenia susceptibility locus on chromosome 1q32 identified in a genetic isolate of Finland. *I. Hovatta[1,2], T. Varilo[1], P. Ellonen[1], R. Arajärvi[2], J. Lönnqvist[2], L. Peltonen[1].* 1) Department of Human Molecular Genetics, National Public Health Institute, Helsinki, Finland; 2) Department of Mental Health and Alcohol Research, National Public Health Institute, Helsinki, Finland.

We have performed a genomewide search for genes predisposing to schizophrenia in a northeastern community of Finland, representing a genetic subisolate inhabited by 40 families some 300 years ago. Ancestors of the studied schizophrenia families with two or more affected were traced back to the 17th century, and 80% of the families were shown to be interrelated via multiple links between ancestors, the most simple links leading to a single founder couple born about 1650. We carried out a genome scan based initially on the monitoring of shared marker alleles among affecteds and subsequently on segregation analyses in core families. We identified four chromosomal regions showing lod scores >1.0 in two-point parametric linkage analysis: 1q32 (Zmax=3.82), 4q31 (Zmax=2.74), 9q21 (Zmax=1.95), and Xp11 (Zmax=2.01). The chromosome 1 region seemed most interesting after the initial scan, not only because of the highest lod scores obtained, but also for a putative 6.6 cM haplotype segregating in four schizophrenia families. Further, an independent sib pair-based genome scan from Finland also provided encouraging lod scores with markers on this region. We have now genotyped altogether 23 markers on about 10 cM region surrounding the haplotype and ordered them in radiation hybrid panels. 20 families with at least two affected siblings and additional 61 families with one affected patient from the isolate were used. We are implying various linkage and linkage disequilibrium based statistical tests to monitor for allelic association and shared chromosomal haplotypes to narrow down the positive region, still farely wide for positional cloning attempts.

Program Nr: 1425 from the 1999 ASHG Annual Meeting

A database of human chromosome 21 SNPs: A tool for association studies to dissect the contribution of minor loci to complex diseases. *A. Imhof, S.E. Antonarakis, H.S. Scott.* Division of Medical Genetics, University of Geneva Medical School, Geneva, Switzerland.

Minor loci for 3 complex phenotypes have been mapped to human chromosome 21 (HC21) by conventional linkage studies including loci controlling Schistosoma mansoni infection intensity (EJHG 1999 7:88), familial combined hyperlipidemia (AJHG 1999 64:1453-63), and bipolar affective disorder (e.g. AJHG 1999 64:210). Several monogenic human disorders have been mapped to HC21 and the genes for some of these disorders identified. Due to its small size (the smallest human chromosome) and medical importance (trisomy 21 causes Down syndrome), HC21 has acted as a model for genomic studies and will be among the first human chromosomes to have its sequence entirely determined (the non-repetitive 21q) as finished genomic sequence. There are already 116 known HC21 genes, mainly telomeric to 21q22.1, a large region the minor loci for the above complex disorders map. Thus an excellent infrastructure exists to identify HC21 SNPs to use in association studies of the diseases above, providing an opportunity to study the efficiency of a high density of SNPs in revealing minor loci for complex diseases. We are using 3 search strategies to identify HC21 specific SNPs by blastn. The following sequences are searched against all human database entries; i) HC21 mRNA sequences, ii) consensus contigs constructed from HC21 STSs identical to HC21 genomic sequence (including ESTs) against all human database entries and iii) HC21 genomic sequence. SNPs are automatically extracted from the blast analyses by a parsing program according parameters for frequency and quality. Putative SNPs and alignments are available at http://medgen.unige.ch/snps/genes1.html. We have already identified 152 putative HC21 SNPs from 60 of the 93 human HC21 genes analysed, 50 from coding regions of the genes (at least 2 independent database entries for each sequence variant). Several SNPs have been confirmed by allele specific oligonucelotide (ASO) hybridization. We are presently testing three SNPs on ASO nitrocellulose coated microarrays as a feasibility study to scale up to a HC21 SNP chip to allow high throughput analyses.

Program Nr: 1426 from the 1999 ASHG Annual Meeting

Mutation in Europe, Polymorphism in China. *M. Jeanpierre[1], Y. Zhang[1,2], J. Forner[1], J.A. Urtizberea[3], H. Cann[4], J.C. Kaplan[1], F. Qishi[2]*. 1) Port-Royal Hospital, Paris, France; 2) Ruijin Hospital, Shanghai, China; 3) Institut de Myologie, Paris, France; 4) CEPH, Paris.

Facioscapulohumeral muscular dystrophy, one of the major forms of muscular dystrophy, is associated with deletions of a 3.3 tandemly repeated sequences (D4Z4) in the subtelomeric region on the long arm of chromosome 4. The link between D4Z4 rearrangement (fragment <28 kb) and the muscular dystrophy is not understood and still a mystery. The proof that the variation of number of repeated sequences is the cause of the disease and not merely an associated polymorphism stems from the concomitant occurrence of de novo D4Z4 rearrangements and a severe clinical course. Since facioscapulohumeral muscular dystrophy is about three time less frequent in Asian populations than European populations, D4Z4 mutation rates should vary in these populations or the consequences of the mutation should not be the same in Europe and Asia. We have studied 155 Shanghai inhabitants and observed short EcoRI fragments (25-28 kb) in 7 individuals, normal upon clinical examination by an experienced neurologist. Further studies made unlikely that these short fragments originate from the homologous chromosome 10 locus. Since the distribution of D4Z4 mutations is skewed toward shorter sizes (<24kb) in Chinese FSHD patients, the frontier between normal and disease alleles must depend on genetic background.

Program Nr: 1427 from the 1999 ASHG Annual Meeting

Molecular analysis of *FBN1*in 3 families with Marfan syndrome. *J.W. Jennings, J.D. Ranells, D.R. Hilbelink, B.G. Kousseff, O.T. Mueller.* Pediatrics/Genetics, University of South Florida, Tampa, FL.

Upon performing a phenotypic analysis of 104 individuals evaluated for MFS for the purpose of re-evaluating the diagnoses in light of the revised criteria for MFS, molecular studies of the fibrillin-1 gene (FBN1) were then performed on 3 families. This involved linkage analysis, heteroduplex mismatch analysis, exonal cloning, restriction digest, and sequencing.

In two families linkage served to identify an affected allele in prepubescent individuals who otherwise did not have the major manifestations of MFS as seen in other family members. In a large MFS family displaying intrafamilial heterogeneity, a novel missense mutation (D757N) was identified in exon 18. The change involved the substitution of a guanine for an adenine at the first position of codon 756, and the aberrant exon was identified in all five affected members by mutation detection analysis, DNA sequencing and by the creation of a novel EcoRI restriction site. The mutation resulted in the substitution of asparagine for the wild type, aspartic acid, in the seventh calcium binding EGF-like domain of the fibrillin-1 protein in a non-consensus region. Likewise, 2 polymorphisms (i.e. exon 18-46 A/G and exon 56 +17 G/C) cosegregated with the affected allele.

In the other family linkage analysis showed that the mother and her two daughters carried the affected FBN1 allele, 3 neutral sequence changes were identified in exon 15, intron 17 and intron 40. No sequence alterations were found. Only one individual from the third family with a definitive diagnosis of MFS was available for testing due to his father's death. Mutation screening revealed no mutations or polymorphisms in the affected individual and his two sons.

These methods isolated a novel mutation and four neutral polymorphisms, indicating that the methods were sensitive enough in identifying sequence alterations. These results support the genetic heterogeneity surrounding the Marfan-like phenotype, suggest that mutations in FBN1 as well as other genes contribute to such, and express the need to locate other genes responsible by linkage and/or candidate gene strategies.

Program Nr: 1428 from the 1999 ASHG Annual Meeting

Identification of seven novel single-nucleotide polymorphisms (SNPs) at the human tissue-type plasminogen activator (t-PA) locus; Association between a polymorphic Sp1 binding site and vascular t-PA release. *C. Jern*[1,2], *P. Ladenvall*[1], *U. Wall*[1], *S. Jern*[1]. 1) Clinical Experimental Research Laboratory, Heart and Lung Institute, Sahlgrenska University Hospital/Ostra, Goteborg University, Sweden; 2) Institute of Clinical Neuroscience, Deptartment of Neurology, Sahlgrenska University Hospital, Goteborg University, Sweden.

We recently reported that an Alu insertion polymorphism of the tissue-type plasminogen activator (t-PA) gene is associated with vascular t-PA release rates in man. Aim of the study was to search for putative functional genetic variants linked with this polymorphism. DNA from healthy individuals with a miscellany of Alu genotypes and t-PA release rates were studied. Upstream regulatory as well as 3-flanking regions were subjected to direct DNA sequencing and coding regions were screened by SSCP analysis. In total, 8.2 kb genomic sequence were examined. Seven single nucleotide polymorphisms (SNPs) were identified. Three of these were in linkage disequilibrium with the Alu polymorphism; one in an upstream regulatory region, one in exon 6, and one in intron 10. The upstream SNP resides within a Sp1 binding motif, and gel shift analysis revealed stronger binding of Sp1 to the C than the T allele. Subjects homozygous for the C allele (the most conserved Sp1 motif) had more than twice the t-PA release rate than subjects carrying the T allele. Variations in exon 6 and intron 10 were silent and without effect on splicing, respectively. Our findings suggest that the newly identified SNP in the Sp1 binding site contributes to the physiological t-PA expression in vivo.

Program Nr: 1429 from the 1999 ASHG Annual Meeting

Characterization of the Chromosome 16p13.1 PXE locus in an Afrikaner population. *E.W. Johnson[1,2], K.A. Curry[1], J.M. Peloquin[1], S.M. Novak[2], C.G. Dokken[2], S.F. Terry[3], L.G. Bercovitch[4], D. Viljoen[5], C.D. Boyd[6]*. 1) Neurogenetics, Barrow Neurological Institute, Phoenix, AZ; 2) Marshfield Medical Research Foundation, Marshfield, WI; 3) PXE International, Sharon, MA; 4) Dept. of Dermatology, Brown University, Providence, RI; 5) SAIMR, Dept. of Human Genetics, Univ. of Witwatersrand, Johannesburg, South Africa; 6) The Pacific Biomedical Research Center, Univ. of Hawaii, Honolulu, HI.

Pseudoxanthoma elasticum (PXE) is a disorder of the connective tissue that shows both autosomal dominant and autosomal recessive inheritance patterns. PXE causes calcification of the elastic fibers mainly effecting the vascular and ocular systems. Previous research has narrowed the locus for a gene for PXE to a region on chromosome 16p13.1 between markers D16s3079 and D16s3103. We have through the efforts of PXE International, obtained DNA from an extended Afrikaner population affected with familial PXE. In this study we attempt to narrow this locus using 15 STRP markers that define the critical interval described above. Initial results indicate that there is a common Afrikaner haplotype in a number of affected individuals from several different families in this population. This common haplotype spans the entire critical interval in many families. The presence of this common affected haplotype indicates that at least some of the affected members of the Afrikaner community shared a common ancestor affected with familial PXE in the not so distant past. To date there is no indication of any ancestral crossover events in the families examined that would aid in narrowing the critical interval where the PXE gene might be found. There are several families where incomplete family collection has limited our ability to define haplotype completely and accurately. Efforts are currently underway to collect DNA from crucial individuals from these families and to define familial haplotype in an effort to uncover informative ancestral crossover events.

Program Nr: 1430 from the 1999 ASHG Annual Meeting

Haplotype analysis of markers on chromosome 2q21.2-q22 in nemaline myopathy in the Amish: Evidence for genetic heterogeneity. *J.J. Johnston[1], R.I. Kelley[2,3], D.H. Morton[2,3], T.O. Crawford[4], L.G. Biesecker[1], C.A. Francomano[1]*. 1) NHGRI, NIH, Bethesda, MD; 2) Department of Pediatrics, Johns Hopkins University, Baltimore, MD; 3) The Clinic for Special Children, Strasburg, PA; 4) Department of Neurology, Johns Hopkins University, Baltimore, MD.

We have previously described an autosomal-recessive form of nemaline myopathy. This disorder of both muscle and central motor function has an incidence of 1 in 500 among the Lancaster County Old Order Amish. The disease has become known as "chicken breast disease" among the Amish because the affected children develop a severe pectus carinatum preterminally. The first signs of the disease appear at birth with excessive jitteriness, ankle clonus, and multiple large joint contractures, although some mothers report abnormal vibratory fetal movements from the 7th month gestation. The jitteriness resolves as the children lose their deep tendon reflexes over the first two to three months. Thereafter they remain hypotonic, but later they develop a rigid pectus carinatum with progressively restricted thoracic movement and die of pulmonary insufficiency in the second to fourth year. Intelligence throughout is normal. Quadriceps muscle biopsy of three children showed disproportionately small type I myofibers with Z-band streaming and abundant nemaline rods. Nebulin is a skeletal muscle protein that is believed to regulate the assembly of Z disks, and nebulin mutations have been identified in families with recessive nemaline myopathy. We have collected DNA from a large Old Order Amish pedigree including samples from seven affected children to perform linkage analysis. Our initial studies have focused on nebulin as a candidate gene for this disorder. Haplotype analysis of several microsatellite markers from the 4-centimorgan region surrounding the nebulin gene on chromosome 2q21.2-q22 has shown recombination between the markers and the disease, excluding nebulin as a candidate gene for this disorder, and demonstrating genetic heterogeneity among families with autosomal recessive nemaline myopathy. We are now focusing on a genome wide screen to identify the gene which is responsible for this disorder in the Amish population.

Program Nr: 1431 from the 1999 ASHG Annual Meeting

Unravelling the genetic complexity of cleft lip in the mouse model, the A/WySn strain. *D.M. Juriloff, M.J. Harris.* Dept Medical Genetics, Univ British Columbia, Vancouver, BC, Canada.

Cleft lip, CL(P), in the A/WySn mouse strain models human CL(P) in genetic complexity and in embryopathology. It is not yet known whether the genetic components are homologs of human genetic CL(P) risk factors. Previously, we mapped a recessive causative gene, *clf1*, to Chr 11 near *D11Mit10*, using a congenic strain. In this study, using a genome screen for linkage on affected segregants after a cross of A/WySn with the normal strain C57BL/6J (1299 first backcross embryos) and using testcrosses of AXB/BXA Recombinant Inbred strains, we have demonstrated an epistatic relationship between *clf1* and a second recessive locus, *clf2*. High risk of CL(P) requires simultaneous homozygosity for "A" strain alleles at both loci. Homozygosity at either locus alone is not sufficient to cause CL(P).

The map position of *clf1* was confirmed to be a 2 cM region between *D11Mit146* and *D11Mit166*, linked to *Neurod2*. The *clf2* locus maps to Chr 13, near *D13Mit13*, linked to *Neurod3*. As both CL(P) loci are linked to paralogs, they may themselves be paralogs in conserved duplicated linkage groups, and functional redundancy may be the origin of the epistasis and complex transmission genetics.

Genetic maternal effects on risk of CL(P) confer another layer of genetic complexity. The prerequisite epistatic genotype for CL(P) in embryos interacts with maternal genotype; A/WySn mothers produce the maximum risk of CL(P). Genetically equivalent first backcross embryos in F1 mothers had no CL(P) in contrast to 2.7 percent in A/WySn mothers ($P<.05$). Reciprocal crosses between A/WySn and AXB-6/Pgn produced 20 percent and 10 percent CL(P) in A/WySn and AXB-6/Pgn mothers, respectively ($P<.05$).

We suggest that a similar combination of epistatic interaction and genetic maternal effects may contribute to the genetic complexity of risk of human CL(P).

Program Nr: 1432 from the 1999 ASHG Annual Meeting

An NdeI polymorphism 3' to the T-cell receptor b **chain gene may be restricted to individuals of African-American ancestry.** *J.A. Kant[1], Z-Y. Chen[1], W.T. Hofgärtner[1], K. Okuyama[1,2].* 1) Dept Pathology, S764B Scaife, Univ Pittsburgh Medical Ctr, Pittsburgh, PA; 2) Dept Obstetrics/Gynecology, Hokkaido University School of Medicine, Sapporo 060, Japan.

T-cell receptor (TCR) b chain gene rearrangement is used to identify clonal T-lineage lymphoproliferative processes using Southern blot analysis with any of a number of restriction endonucleases. The enzyme NdeI is a useful enzyme in these studies because it detects rearrangement into both b D-J-C loci and produces a modestly smaller germline restriction fragment of 21.0 kb than the more-commonly used BamHI. We recently reviewed a consecutive series of 337 patient samples analyzed with NdeI, 7 of which demonstrated an apparent polymorphism as an additional 27 kb germline fragment. This is a useful observation for investigators performing clonality assays to avoid a possible misdiagnosis. All samples demonstrating the polymorphism for which information about ethnic background was available (6 of 7) came from individuals of African-American descent. We have confirmed by direct DNA sequencing of both strands in all samples demonstrating the 27 kb germline fragment that the polymorphism results from a G>A transition at nucleotide 206319 of the Genebank sequence U66061. Studies are underway to define the distribution of this polymorphism in a broader series of African-American, Hispanic and Asian samples.

Program Nr: 1433 from the 1999 ASHG Annual Meeting

Genetic studies on Finnish families with familial hemiplegic migraine. *M. Kaunisto[1,2], M. Kallela[1], P. Marttila[3], M. Färkkilä[1], H. Havanka[4], E. Hämäläinen[2], I. Hovatta[5], A. Orpana[2], L. Peltonen[3], A. Palotie[3], M. Wessman[1,2].* 1) Departments of Biosciences and Neurology, University of Helsinki, Helsinki, Finland; 2) Laboratory of Helsinki University Central Hospital, Helsinki, Finland; 3) Departments of Pathology and Human Genetics, UCLA, LA, USA; 4) Länsi-Pohja Central Hospital, Kemi, Finland; 5) Department of Human Molecular Genetics, National Public Health Institute, Helsinki, Finland.

Familial hemiplegic migraine (FHM) is an autosomal dominant subtype of migraine with aura. Patients with FHM have migraine attacks which are associated with hemiparesis or hemiplegia. In addition, some patients have progressive cerebellar ataxia. The gene for approximately a half of the reported FHM-families has been localized to chromosome 19p13. This gene, *CACNA1A*, encodes the a1A subunit of a P/Q-type voltage-gated calcium channel. 5 different missense mutations in *CACNA1A* (in FHM) have been published so far. FHM has shown to be a genetically heterogeneous disease after 2 groups found linkage to chromosome 1q.

We studied 9 families (71 members) fulfilling the IHS diagnosis criteria for FHM. These families originate from a database of 255 Finnish migraine families. Altogether 25 subjects were classified as affected: they fulfilled the IHS criteria for migraine with aura and had some degree of hemiparesis with their attacks. We analyzed the families for linkage to 3 chromosomal areas (19p13, 1q21-23 and 1q31) previously linked to FHM. 3 of the 9 families gave suggestive evidence for linkage to 19p13 showing positive lod scores with several markers. The total maximum LOD score was 3.3. For these families haplotypes segregating with the disease were determined. This data gave evidence against a major founder effect. Evidence for linkage to chromosome 1q was not obtained. We are currently screening these families for mutations in *CACNA1A* gene. So far no mutations have been found in 15/47 exons. These 15 exons include all previously published FHM mutation sites. However, we observed 4 previously described polymorphisms. Our data indicates that the Finnish FHM-mutations at least in these families are different from the previously published ones.

Program Nr: 1434 from the 1999 ASHG Annual Meeting

Refinement of the locus for Autosomal Recessive Retinitis Pigmentosa (RP25) linked to Chromosome 6q in a family of Pakistani Origin. *S. Khaliq[1,2], A. Hameed[2], M. Ismail[2], S.Q. Mehdi[2], D.A.R. Bessant[1], A.M. Payne[1], S.S. Bhattacharya[1]*. 1) Molecular Genetics, Inst. Ophthalmology, London, London, UK; 2) Research Laboratories, Biomedical and Genetic Engineering Division, Islamabad, Pakistan.

Retinitis pigmentosa (RP) is a clinically and genetically heterogeneous group of retinal degenerations primarily affecting the rod photoreceptors. RP is characterised by progressive loss of vision, initially manifesting as night blindness and reduction in the peripheral visual field, and later involving loss of central vision (Bird 1985). Recently linkage of arRP to a region on chromosome 6q has been reported in several Spanish families (Ruiz et al. 1998) We studied 20 members of a 3 generation consanguineous Pakistani family with arRP. To identify the locus responsible for disease in this family we performed homozygosity analysis. Significant linkage was obtained for 3 markers on chromosome 6q in region of a previously reported locus (RetNet). A maximum lod score value of 3.30 at q. = 0.00 was observed for markers D6S257 and D6S1053 on chromosome 6q. Recombination events involving the centromeric marker D6S1053 and the telomeric marker D6S430 permitted refinement of the 6q arRP (RP25) disease critical region from the previously reported 16.1cM (Ruiz et al. 1998) to 2.4cM.The GABA receptor candidate genes were proposed as candidates for this phenotype by Ruiz et al. (1998) in their initial linkage paper. The recombination events seen in our family exclude both GABRR1 and GABRR2 as the disease gene and the area of homozygosity observed by Ruiz et al. We cannot however exclude the possibility that there are in fact two arRP loci located in close proximity on 6q, one in the Spanish population and one in the Pakistani population. The 2.4 cM critical interval contains no well characterised candidate genes, but numerous ESTs. Further analysis of these cDNA clones will be needed before mutation screening in this family can be undertaken. The identification of a family of Pakistani origin in addition to the 5 Spanish families suggests that this may be an important gene for arRP.

Program Nr: 1435 from the 1999 ASHG Annual Meeting

Identification of new microsatellite markers on chromosome 4p16. *G. Kirov, M.J. Owen.* Neuropsychiatric Genetics Unit, UWCM, Cardiff, UK.

Chromosome 4p16 has been implicated as a candidate region in psychiatric genetics due to several positive reports from linkage studies in manic depressive illness and schizophrenia. The region is being sequenced by Stanford Human Genome Center and the raw data is made freely available to the genetic community. The availability of raw unfinished sequence data allows the identification of new polymorphisms in this area. We present our approach to identifying such polymorphisms.

Methods: Firstly, we examine sequenced BAC clones with the programme SPUTNIK (http://www.abajian.com/sputnik) which identifies di-, tri, tetra- and pentanucleotide repeats. It assigns a score to each repeat based on its length and how perfect the repeat is. Thirty-eight primer pairs flanking the promising repeats were designed, based on available sequence, and 30 of those amplified successfully. In order to assess whether a marker is polymorphic we analyse only two individuals and the products from a pool from 60 individuals. In most cases this was sufficient to determine whether the marker is polymorphic, as well as to assess the range of alleles and make a judgement about its heterozygosity.

Summary of results: We have examined so far 9 BAC clones which cover an area of ~1.5Mb between markers D4S827 and D4S2983 and attempted to place at least two markers per clone. This achieves an approximate saturation of one marker at every ~100,000 bp with no gaps longer than ~150,000 bp (The size of the clones varied between 150,000 and 200,000 bp). Seven microsatellites were already available in GDB. Our method identified 12 additional markers with a heterozygoisity of 0.2 or higher. As expected, CA repeats were more likely to be polymorphic but we also identified three tetra- and one trinucleotide repeat with four or more alleles. This study confirms that it is possible to saturate this region of interest with microsatellite markers spaced at an average of 100,000 bp which will allow linkage disequilibrium mapping. Our protocol allows for one research worker to screen ~1Mb of sequence in one to two weeks.

Program Nr: 1436 from the 1999 ASHG Annual Meeting

Genetic linkage of adult onset primary open angle glaucoma to chromosome 3q in a Greek pedigree. *G. Kitsos[1], H. Eiberg[2], E. Economou-Petersen[3], M.K. Wirtz[4], P.L. Kramer[4], M. Aspiotis[1], N. Tommerup[2], M.B. Petersen[5], K. Psilas[1]*. 1) Dept Ophthalmology, Univ Ioannina, Ioannina, Greece; 2) Inst Med Biochem Genet, Univ Copenhagen, Copenhagen, Denmark; 3) Drakopoulion Blood Bank Center, Athens, Greece; 4) Oregon Health Sciences Univ, Portland, Oregon; 5) Inst Child Health, Athens, Greece.

A locus for juvenile onset primary open angle glaucoma (POAG) was assigned to chromosome 1q in families with autosomal dominant inheritance (GLC1A), due to mutations in the TIGR gene. For adult onset POAG, five loci have so far been mapped by linkage analysis in families with autosomal dominant mode of inheritance and age-dependent penetrance: GLC1B to 2cen-q13, GLC1C to 3q21-q24, GLC1D to 8q23, GLC1E to 10p15-p14, and GLC1F to 7q35-q36, showing the high degree of genetic heterogeneity in POAG. Except for the GLC1B locus, the other loci for adult onset POAG have so far been reported only in single large pedigrees, and their contribution to POAG worldwide has therefore not been established. We have studied a large family identified in Epirus, Greece, segregating adult onset POAG in an autosomal dominant fashion. Fifteen affected family members were identified from 40 at-risk individuals above 30 years of age (75% penetrance). Clinical findings included increased cup to disc ratio, characteristic changes in the visual field, and untreated intraocular pressure more than 21 mmHg, with age at diagnosis 30 years and older. Linkage was established with a group of DNA markers located on 3q, where the GLC1C locus previously had been located. Multipoint linkage analysis between the disease phenotype and microsatellite DNA markers located on chromosome 3q gave a maximum lod score of 4.22 for marker D3S1763. This represents the second adult onset POAG family linked to the GLC1C locus on chromosome 3q, and microsatellite analysis in the two families demonstrated no allele sharing within the most likely region of linkage, indicating that the genetic defect most probably was of independent origin.

Program Nr: 1437 from the 1999 ASHG Annual Meeting

Recombination breakpoint analysis for autosomal dominant exudative vitreoretinopathy (FEVR) gene. *H. Kondo[1], H. Hayashi[2], K. Oshima[2], T. Tahira[1], K. Hayashi[1].* 1) Division of Genome Analysis, Inst. Genet. Information, Fukuoka, Fukuoka, Japan; 2) Department of Ophthalmology, Fukuoka University School of Medicine, Fukuoka, Japan.

Autosomal dominant exudative vitreoretinopathy (FEVR) is an ocular disorder that affects the development of retinal vasculature and causes retinal detachments in the youth as well as retinal folds in infants. Many large families with FEVR were identified especially in the Netherlands and Japan, but linkage analysis has not been reported in Japanese family. FEVR gene locus was previously localized in 11q13-23 and closely linked to microsatellite markers D11S533 and D11S873 in European and Asian families of unidentified ethnic origin. However, the region of FEVR gene locus has not been delineated. In the present study, seven microsatellite markers including two markers above in 11q13 were analyzed in 37 individuals who belong to eight Japanese FEVR families. Two-point linkage analysis revealed that FEVR was linked to all markers in seven out of eight families although the combined LOD scores were not significant (Zmax = 2.4 at theta = 0). Next, alleles in the seven families were haplotyped according to the parent of origin. The disease haplotype was preserved in all affected individuals in the six families. However, in the seventh family, the centromeric recombination was found in one affected individual at most proximal marker (D11S1314) while telomeric recombination was found in another affected individual at the second distal marker (D11S873). Thus the critical region was narrowed to 15.2 cM. Analyses are in progress to further narrow the region.

Program Nr: 1438 from the 1999 ASHG Annual Meeting

Genomic screen for linkage of May-Hegglin anomaly. *J.F. Korczak[1], W. Jawien[2], A.Y. Lin[3], K. Hoffmeister[2], M.J. Kelley[4]*. 1) Georgetown Univ Med Ctr, Washington, DC; 2) National Naval Med Ctr, Bethesda, MD; 3) Santa Clara Valley Med Ctr, Santa Clara, CA; 4) Duke Univ Med Ctr, Durham, NC.

May-Hegglin anomaly (MHA; MIM#155100), an autosomal dominant platelet disorder, is characterized by the triad thrombocytopenia, giant platelets, and inclusion bodies in the leukocytes. About 30% of affected individuals have mild to moderate hemorrhagic symptoms and are frequently misdiagnosed and subjected to inappropriate treatments. Although about 300 cases have been reported, the genetic defect for MHA has not been identified, nor have functional studies revealed its etiology.

We evaluated three families with multiple cases (9, 2, and 2) of MHA in several generations, with affected individuals evenly distributed across genders (7 males and 6 females), and performed a genomic screen for linkage to identify chromosomal region(s) to which the MHA locus may map. Sufficient power to detect linkage was demonstrated by the program SIMLINK. Based on 200 replicates, the estimated mean and maximum lod scores at a recombination fraction of 0.05 (for a 10 cM map) were 3.3 and 6.4, respectively, assuming a rare, autosomal dominant gene with complete penetrance. DNA from 34 individuals (12 of 13 with MHA) was genotyped for 386 markers spaced 10 cM apart of the CHLC version 9 set at the Center for Inherited Disease Research (CIDR). Nonparametric two-point sib-pair linkage methods (affected sib-pair (ASP) and Haseman-Elston regression (H-E)), implemented in the S.A.G.E. (3.1) program SIBPAL, were used to test for linkage. Possible regions containing the MHA gene were suggested on chromosomes 3, 10, and 22, based on nominal *P*-values £ 0.01 for two or more adjacent markers for the ASP and/or H-E tests.

We are performing two-point lod score analysis to corroborate our findings and genotyping additional markers in regions of interest for multipoint analysis. We have also ascertained additional multiplex families to fine-map the MHA gene prior to undertaking a positional candidate approach to identify it.

Program Nr: 1439 from the 1999 ASHG Annual Meeting

Towards positional cloning of the Meckel Syndrome gene. *M. Kyttala[1,2], P. Paavola[2], R. Salonen[3], L. Peltonen[1,2].* 1) Human Genetics, University of California L.A., Los Angeles, CA; 2) Department of Human Molecular Genetics, National Public Health Institute, Helsinki, Finland; 3) Department of Obstetrics and Gynecology, Helsinki University Hospital, Finland.

Meckel Syndrome (MKS; MIM 249000) is an autosomal resessive congenital malformation syndrome leading to death soon after birth. The clinical phenotype of MKS includes central nervous system malformations, occipital meningo-encephalocele, polycystic kidneys, cystic and fibrotic changes in the liver, and polydactyly. In Finland the prevalence of MKS at birth is estimated to be 1:9000. MKS has been mapped in Finnish population into chromosome 17q22-23, but the locus heterogenity in study samples from other populations has also been reported with a linkage to chromosome 11q13 (Roume et al., 1998). We have restricted the critical chromosomal region on 17q to 700 kb using linkage disequilibrium and ancient haplotype in Finnish disease alleles. We have built a multiple coverage physical contig of PAC and BAC clones over this region and ordered them using visual mapping by fiber FISH, as well as end sequencing of the clones to develop new STS`s for screening of clones and securing the overlaps between the clones. There are total of 10 known genes and 30 EST contigs in databases in this restricted DNA region. Complete information of multiple genomic clones on the region is also available from genome databases. We isolated novel CA repeats from these clones and two of them provided almost complete allelic assosciation with the MKS (lambda:0,98; p-value<10-15) and suggests an immediate vicinity of MKS gene to these markers located 70 kb apart from each other. This information provides now basis for the final isolation of the MKS gene. We are currently sequencing coding regions of genes and EST contigs in Finnish MKS patients to search for MKS-associated DNA variations.

Program Nr: 1440 from the 1999 ASHG Annual Meeting

Multiple Trait Locus Nonparametric Linkage Regression. *C.D. Langefeld, M. Boehnke.* Biostatistics, Sch Public Hlth, Univ Michigan, Ann Arbor, MI.

Complex genetic traits may exhibit incomplete penetrance, phenocopies, heterogeneity and polygenic inheritance. Thus, improving the efficiency of linkage methods in the localization of complex disease loci and in the placement of loci and environmental factors into functional groups, may require methods that jointly consider the effects of genetic heterogeneity, epistatis and environmental factors. We propose and examine the performance of a class of nonparametric (mode-of-inheritance free) linkage (NPL) methods based on conditional logistic regression. The primary advantage of this regression approach is that it easily extends to tests of an arbitrary number of loci, other phenotypic variables, environmental factors and their interactions. For independent affected sibling pairs (ASP), we show that when the proportion of alleles shared identical by descent (IBD) is observed, the ASP mean test and the score test from a one-locus conditional logistic regression model are asymptotically equivalent tests of linkage. When the IBD proportion is observed, the single- and multiple- locus regression models have the appropriate type I error rates. When the IBD proportion is estimated, the type I error rates of the large-sample tests can be larger than the nominal level; under these conditions, we propose a permutation test. Whittemore and Halpern (1994) proposed a general class T of NPL tests based on scoring functions of all possible inheritance vectors configurations of the observed marker genotypes within a pedigree. This class of tests does not require that the mode of inheritance be specified or the IBD proportions be observed. We show that the single-locus score test from conditional logistic regression is asymptotically equivalent to T. In simulations of two popular members of T implemented in GENEHUNTER, NPL(pairs) and NPL(all), the single-locus NPL regression model have appropriate type I error rates. We discuss and illustrate extensions to multiple loci, other phenotypic variables, environmental factors and their interactions.

Program Nr: 1441 from the 1999 ASHG Annual Meeting

Promoter sequence studies of the *LGALS3* (galectin-3) gene in insulin-dependent diabetes mellitus. *Z. Larsen, O.P. Kristiansen, J. Johannesen, J. Nerup, F. Pociot.* Steno Diabetes Center, Gentofte, Denmark.

Galectin-3 is a b-galactoside-specific lectin found in many species and cell types. The gene is located on the long arm of chromosome 14 (14q21). Results from genome-wide screenings and partial ones have showed some evidence for linkage in the Galectin-3 gene region, *D14S276*. In the present study we have screened for polymorphisms in the promoter region and the intron 2 of the Galectin-3 gene which also has showed promoter activity. Combining SSCP (Single Stranded Conformation Polymorphism) and cycle sequencing methods we have identified 3 polymorphisms within 60 bp of the promoter region. Two of the polymorphisms are single base substitutions leading to different restrictions sites (*MseI* and *StuI*). The third polymorphism is a 12 bp deletion which also lead to a different restriction sites (*Eco0109I*). All three polymorphisms have high heterozygosity and were analysed for linkage to IDDM in a large homogeneous Danish family material comprising 251 families (1041 individuals) with 410 IDDM-affected offspring. Transmissions of wildtype (W) and mutant (M) alleles from heterozygous parents to offspring are shown in the table below:

	IDDM W/M	non-IDDM W/M
MseI (-715)	154/140	105/98
Eco0109I (-698)	160/157	100/114
StuI (-659)	106/110	56/66

The numbers in brackets are the distance from the transcription site. Even though Galectin-3 is a very promising candidate gene for IDDM we found no linkage to IDDM of the identified polymorphisms. An etiological mutation may still be found in the coding region of the gene, the 3UTR or combinations leading to specific haplotypes.

Program Nr: 1442 from the 1999 ASHG Annual Meeting

Genetic heterogeneity of Paget's disease of bone: exclusion of linkage to the *PDB1* and *PDB2* loci in French-Canadian pedigrees. *N. Laurin[1], J. Brown[2], A. Duchesne[1], C. Brousseau[2], D. Huot[2], Y. Lacourcière[2], G. Drapeau[2], J. Verreault[2], V. Raymond[1], J. Morissette[1]*. 1) Molecular Endocrinology, CHUL Research Center, Ste-Foy, Qc, Canada; 2) Immunology and Rhumatology, CHUL Research Center, Ste-Foy, Qc, Canada.

Paget's disease of bone (PDB), characterized by high bone remodeling and large, multinucleated osteoclasts, is transmitted as an autosomal dominant trait in some families. Two PDB disease loci: *PDB1* and *PDB2*, have been mapped to chromosome 6p21.3 and 18q21.1-q22, respectively. We reported previously that 4 French-Canadian PDB families were not linked to *PDB2*. Genetic linkage analysis has now been extended to 12 additional families with at least 2 affected first degree relatives. Diagnosis of PDB was based on total serum alkaline phosphatase, total body bone scan and skull and pelvis x-rays. Amongst the 552 individuals who have been recruited, 123 were diagnosed PDB, 265 normal and 164 unknown. Four Généthon markers spanning *PDB2* (D18S64 to D18S68) were selected. The largest pedigree (2 families) was also genotyped with 8 additional markers extending this region to 18q12.2-q23 (D18S1157 to D18S1161). No support for linkage as well as no characteristic "disease" haplotype were observed in any of the families tested. Negative lod score values below -2.00 were calculated for each of the 6 largest pedigrees. A partial genome scan was then performed with 7 selected individuals and markers spanning candidate loci for bone traits/disorders: chromosome 1 (low mineral density), 5 (craniometaphyseal dysplasia), 8 (multiple exostoses), 9 (diaphyseal medulary stenosis), 10 (spondyloepimetaphyseal dysplasia), 11 (high bone mass), 17 (Van Buchem) and 22 (minor histocompatibility Ag). None of these regions remained as candidate. A complete genome scan using Généthon markers is now in progress. Linkage to chromosome 2 and *PDB1* at 6p has already been excluded in the 3 most informative families. Our data shows that the gene(s) responsible for PDB in the French-Canadian population is/are not located at *PDB1*, *PDB2* nor on chromosome 2, demonstrating genetic heterogeneity of the disorder.

Program Nr: 1443 from the 1999 ASHG Annual Meeting

Myostatin promoter sequence and frequent allelic variation in the coding sequence. *E.C. Lawrence[1], B.F. Hurley[2], S.M. Roth[2], J.M. Hagberg[2], R.E. Ferrell[1]*. 1) Human Genetics, University of Pittsburgh, Pittsburgh, PA; 2) Dept. of Kinesiology, University of Maryland, College Park, MD.

Myostatin, also known as growth and differentiation factor 8, is a recently identified member of the transforming growth factor-beta family of regulatory molecules. Mutations in this gene caused the double-muscled phenotype in cattle and "compact" hypermuscular phenotype in mice. We ascertained a sample of Caucasian (N=107) and African American (N=41) individuals who had responded to strength training with a significant increase in muscle mass, and a second group of individuals (N=48) who were classified as non-responders on the basis of an undetected increase in muscle mass with strength training. The study sample was sequenced for the three myostatin exons and the promoter region. The coding sequence contained five missense substitutions in amino acid residues that are conserved in all mammalian myostatins sequenced to date (A55T, K153R, E164K, P198A, and I225T). Two of these, A55T in exon 1 and K153R in exon 2, are polymorphic in both the study population and in a random sample of Caucasians (N=96) and African Americans (N=96), and both the study group and the random sample showed significantly different allele frequencies between the Caucasian and African American individuals ($p<0.001$). Neither of the common polymorphisms was significantly associated with muscle mass response to strength training in either Caucasians or African Americans, although skewed allele frequencies preclude detection of small effects. No variation was detected in the myostatin promoter. The promoter contains a consensus MyoD response element at position -185, consistent with myostatin acting downstream of the myogenic transcription factor, MyoD, in muscle development.

Program Nr: 1444 from the 1999 ASHG Annual Meeting

Linkage Analysis of Calcium Channel Genes in Typical Migraine Families. *R.A Lea[1], D.R Nyholt[2], R.P Curtain[1], K.L Jordan[1], L.R Griffiths[1]*. 1) Genomics Res Ctr, Sch Hlth Sci, Griffith University-Gold Coast, Southport QLD, Australia; 2) Laboratory of Statistical Genetics, Rockefeller University, New York.

Migraine is a prevalent neurological disorder characterised by recurrent attacks of debilitating headache. The disease is genetically heterogeneous although the type and number of genes involved is still unknown. Mutations in the neuronal calcium channel gene (CACNA1A) have been shown to cause FHM, a rare and severe subtype of migraine [1]. Independent linkage analyses suggest that there are two FHM loci on chromosome 1 near the calcium channel genes CACNA1E and CACNA1S [2,3]. These findings suggest that calcium channel genes may also be involved in the more common types of migraine (with and without aura). We have previously reported linkage to CACNA1A in a large typical migraine family [4] and are currently performing linkage analyses on markers located in other calcium channel gene regions. 108 migraine families including 8 large multigenerational pedigrees have been genotyped for markers spanning the FHM implicated loci on chromosome 1 and results analysed using the GENEHUNTER-PLUS program. Although one large multigenerational family displayed some excess allele sharing, multipoint analysis of combined results significantly excluded the markers surrounding CACNA1S and CACNA1E (LOD < 2). These results indicate that if this locus plays a role in migraine it probably contributes only a small genetic effect to the disease. Other neuronal calcium channel gene markers located across the genome are currently being investigated in our migraine families. 1. Ophoff et al (1996). Cell;87:543-552 2. Gardener et al (1997). Neurology;47:1231-1238 3. Ducros et al (1997). Ann Neurol;42:885-890 4. Nyholt et al (1998). Neurology;50:1428-1432.

Program Nr: 1445 from the 1999 ASHG Annual Meeting

Fine-mapping and mutational analyses of cerebrotendinous xanthomatosis in US pedigrees. *M.H. Lee[1], S. Yi[1], J.D. Carpten[2], J. Cohen[3], G. Salen[4], G.T. Gerhardt[5], S. Patel[1]*. 1) Medicine, Medical University of SC, Charleston, SC; 2) Prostate-Cancer Investigation Group Laboratory of Cancer Genetics, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; 3) University of Texas Southwestern Medical Center, Dallas, TX; 4) University Medical and Dental New Jersey, VA Hospital, East Orange, NJ; 5) Oregon Health Sciences University Department of Medicine, Portland , OR.

Cerebrotendinous xanthomatosis (CTX) is a rare Autosomal Recessive disorder of bile acid biosynthesis. Clinically, CTX patients present with tendon xanthomas, juvenile cataracts, progressive neurological dysfunction and can be diagnosed by the detection of elevated plasma cholestanol levels. CTX is caused by mutations affecting the sterol 27 hydroxylase gene. CTX patients have been identified in a number of populations, but seems to be more prevalent in the Japanese, Sephardic Jewish and Italian populations. We have assembled 12 previously unreported pedigrees and one other proband from the US. The CYP27 locus had been previously mapped to chromosome 2q33-qter. We initially fine mapped this locus to a YAC contig and used a set of flanking microsattelite markers to genotype all of the biochemically diagnosed patients with CTX. All CTX patients showed segregation with the CYP27 locus, suggesting that there is no genetic heterogeneity for this rare phenotype. Haplotype analyses and recombinant events allowed us to precisely map CYP27 between markers D2S1371 and D2S433. Fifteen mutations were identified from 9 probands analyzed thusfar; 8 had heterozygous mutations and one had a homozygous mutation. Of these, seven are novel mutations (Arg94Tyr, Arg94Gln, Tyr100Stop, Lys226Arg, Pro408Cys, Gln428Stop and a 10 bp deletion in exon 1 of CYP27 gene). In summary, we have fine mapped the CYP27 locus, identified 7 more mutations and structure-function studies are underway to correlate phenotype-genotype.

Program Nr: 1446 from the 1999 ASHG Annual Meeting

Genome wide linkage analysis for vacuolating leukoencephalopathy. *P.A.J. Leegwater[1,2], A.M. Könst[1], J. Mulders[1,3], C.B.M. Oudejans[1], R.B.H. Schutgens[1], J.C. Pronk[3], M.S. Van der Knaap[2].* 1) Clinical Chemistry, Academic Hospital; 2) Child Neurology, Academic Hospital; 3) Human Genetics, Medical Faculty, Free University, Amsterdam, The Netherlands.

Vacuolating leukoencephalopathy (VLE) is a pediatric neurologic disorder with macrocephaly and progressive cerebellar ataxia. Magnetic resonance imaging of the brain shows impressive abnormalities of the cerebral white matter which appears to be diffuse and swollen. Subcortical cysts are always present. Histopathology demonstrates the presence of numerous vacuoles between the outer lamellae of myelin sheets of the central nervous system. VLE follows autosomal recessive inheritance and the disease occurs in a variety of ethnic groups. We have performed a genome wide scan for linkage between VLE and microsatellite markers with an average spacing of 11 cM. Only 3 affected children from different first-cousin marriages were typed in the first round of analysis. Genomic regions that are identical by descent in these patients can be expected to span 20 - 30 cM. Therefore, we scored a patient positive for a region when at least 2 adjacent markers were homozygous in that region. Regions that were indicative for linkage in at least 2 of the 3 patients were analysed in additional patients. A similar strategy proved to be of great value for the localization of another childhood neurologic disorder. Eleven candidate regions were evaluated in the expanded population of 15 patients from 10 families. These families are from varying ethnic origin. Thus far, we have eliminated 7 of the 11 candidate regions. The remaining candidate regions around D2S1777, D4S1644, D17S122 and D21S1432 are further investigated with closely spaced markers.

Program Nr: 1447 from the 1999 ASHG Annual Meeting

Protective effect of CYP2D6*4 allele in Ulcerative Colitis. *S. Lesage[1,2], M. Chamaillard[1,2], JF. Colombel & EPIMAD[2], J. Belaiche & the GETAID[2], JP. Cezard & EPGWGPIBD[2], C. Tysk[2], S. Almer[2], M. Gasull[2], H. Zouali[1,2], J. Macry[2], G. Thomas[1,2], JP. Hugot[1,2].* 1) Fondation Jean Dausset, Paris, France; 2) European Concerted Action on the Genetics of the Inflammatory Bowel Diseases.

Background : The CYP2D6 enzyme (debrisoquine-hydroxylase) has a role in the metabolism of a wide range of drugs. The main polymorhism allele associated with poor metaboliser phenotype is CYP2D6*4 which is characterised by a mutation in the splicing site : 1934 G/A. An association has been recently described between CYP2D6*4 allele and Ankylosing Spondylitis (AS). This observation prompted us to analyse this polymorphism in Inflammatory Bowel Diseases (IBD) which are known to be associated with AS.

Aim of the study : To determine whether CYP2D6*4 allele is associated with IBD.

Patients and methods : A total of 395 IBD families with at least one affected child were genotyped for the 1934 G/A polymorphism. Out of these families, 247 had only Crohns Disease (CD) patients, 56 had only Ulcerative Colitis (UC) patients, and 92 mixed families had both CD's and UC's patients. The DNA samples from family members were typed by a PCR-RFLP method. Genotyping data were analyzed using a transmission disequilibrium test (TDT-like from the Analyse package).

Results : The CYP2D6*4 allele was less often transmitted to UC's patients than expected by chance (14 transmitted alleles versus 36 ; $p = 0.002$). In contrast, the CYP2D6*4 allele was equally transmitted to the CD patients (90 transmitted alleles versus 96 ; $p = 0.660$) and healthy relatives (106 transmitted alleles versus 100 ; $p = 0.676$).

Conclusion : The CYP2D6*4 allele may have a protective effect for UC. It suggests that a CYP2D6 normal metabolism pathway seems to be necessary to develop the disease.

Program Nr: 1448 from the 1999 ASHG Annual Meeting

Multicenter linkage study of schizophrenia candidate regions on chromosomes 5q, 6q, 10p and 13q. *D.F. Levinson.* Dept Psychiatry, University of Pennsylvania, Philadelphia, PA.

From the Schizophrenia Linkage Collaborative Group III (DF Levinson, SE Antonarakis, JL Blouin, PV Gejman, P Holmans, KS Kendler, C Laurent, BJ Mowry, MJ Owen, AE Pulver, AR Sanders, SG Schwab, RE Straub, DB Wildenauer, N Williams and collaborators). A multicenter sample has been formed for a series of linkage studies of schizophrenia candidate regions. The eight clinical samples have been collected by the seven collaborating groups (at University of Bonn, University of Chicago, Johns Hopkins University, LGN/CNRS, Virginia Commonwealth University, University of Queensland/University of Pennsylvania/Mt. Sinai Medical School/University of Iowa, and University of Wales) and by the NIMH Schizophrenia Genetics Initiative. In the present study, 32 markers within candidate regions on chromosomes 5q, 6q, 10p and 13q were genotyped in a total of 859 pedigrees including more than 1,900 typed individuals affected with schizophrenia or schizoaffective disorder, with an average of 970 affected sibling pairs typed per marker. Planned analyses include multipoint NPL and likelihood-based affected sibling pair analyses for each sample and for the combined sample using Genehunter 2.0 (based on separate allele frequency estimates for each sample), and a logistic regression analysis incorporating sample as a covariate to estimate the significance of heterogeneity as well as overall marker allele sharing. Preliminary NPL and ASP analyses have been completed for the eight individual samples. Different patterns of results have been observed across the four regions: the number of samples producing an NPL score > 1.0 was 4, 5, 5 and 1 respectively for chromosomes 5q, 6q, 10p and 13q; the number of samples producing an NPL score > 2.0 was 1, 2, 3 and 1 respectively; while an NPL score > 3.0 was observed for one sample for chromosome 6q and one sample for chromosome 13q. Interpretation of the results should await analyses of the combined sample, as well as the logistic regression analysis. The complete analysis will be available for presentation at the meeting.

Program Nr: 1449 from the 1999 ASHG Annual Meeting

Use of the internet for family study recruitment. *K.L. Levinson, R.E. Ferrell, D.N. Finegold.* Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA.

Recruitment of large families with rare genetic diseases is arduous. The internet as a recruitment method for family studies offers advantages to both participants and researchers. We report on the use of a world wide web page (www.pitt.edu/~genetics/lymph) to disseminate information about primary lymphedema, solicit participation in a lymphedema linkage study, and update participants on the progress of the research. In the 675 days since the creation of the web page on August 22, 1997, there have been 16,660 hits to the site, with an average of 25 hits per day. Of the 84 families recruited into the study, 24 were recruited before the web page was created, and 40 of the remaining 60 families were recruited from the internet (67%). Lymphedema Family Study participants are located in 47 US states and 6 Canadian provinces as well as Australia, New Zealand, France, and England. Individuals recruited into the study have provided DNA samples leading to the identification of vascular endothelial growth factor receptor-3 as a causative gene for primary lymphedema. Further analysis of families unlinked to 5q34-35 will ultimately lead to the identification of additional causative genes.

Similar approaches may be useful for other research groups. Researchers may use the internet to reduce the labor intensive contact of multiple uninterested families, while considerably expanding the pool of potential subjects from which to recruit. Participants benefit from initial anonymity by requesting information about the study without disclosing any personal identifiers. Drawbacks do exist, however, since families at a distance have no opportunity for personal contact with the researchers, and many individuals have no access to the internet. While the internet appears suitable for recruitment of families for linkage studies aimed at identifying disease-causing genes, families recruited by this mechanism are likely to exhibit more severe phenotypes or highly penetrant mutations. Affection status, particularly for conditions with variable expressivity, may require confirmation by medical records or physician examination.

Program Nr: 1450 from the 1999 ASHG Annual Meeting

Dominantly inherited myasthenia gravis as a separate genetic entity unlinked to the acetylcholine receptor subunit genes and collagen-like tail subunit gene. *F.Y. Li[1], A. Szobor[2], S. Komoly[2], R. Croxen[3], D. Beeson[3], C. Larsson[1]*. 1) Department of Molecular Medicine, CMM L8:01, Karolinska Hospital, S-171 76 Stockholm, Sweden; 2) Department of Neurology, Jahn Ferenc Teaching Hospital, Semmelweis University Medical School Affiliate, Kves u. 2-4, Budapest-1204, Hungary; 3) Neurosciences Group, Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford OX3 9DS, U.K.

Myasthenia gravis (MG) is an organ specific autoimmune disease characterized by muscular weakness and autoantibodies against the nicotinic acetylcholine receptor (AchR). MG without autoantibody against AchR does also exist. Mutation analysis indicates that the changes of AchR subunit genes, CHRNA1 (2q24-q32), CHRNB1 (17p12-11), CHRNE (17p13-p12), CHRNG (2q21.1-21.3) or collagen-like tail subunit (COLQ) (3p24.3-3p25) are responsible for congenital myasthenic syndrome (CMS). MG occurs in both sporadic and familial forms. The vast majority of MG cases are sporadic, although familial forms are reported in up to 4 % of the cases. We present here a large Hungarian family where nine members from two generations are affected by MG. In order to establish weather MG in this family represents a separate genetic entity or is a variant linked to any of the genes known to be mutated in CMS, the family members were genotyped with microsatellite markers covering the candidate regions. Multipoint linkage analysis gave the lod scores smaller than -2 for makers within those regions. Thus, those genes are excluded as candidate genes for this family and MG in this family represents a separate genetic entity. Establishment of the underlying genetic defect in this family will add to the knowledge of MG pathogenesis.

Program Nr: 1451 from the 1999 ASHG Annual Meeting

The Additive Genetic Gamma Frailty Model for Linkage Analysis of Age-at-onset Variation for Complex Diseases. *H. Li.* Rowe Program in Human Genetics, University of California, Davis School of Medicine, Davis, CA.

Age of onset is a key factor in the linkage analysis of many complex diseases. Current methods in nonparametric linkage analysis are mainly concentrated on the affected relative pairs or affected family members with age of onset information either ignored or taken into account by specifying age-dependent penetrances for liability classes. On the other hand, gamma frailty models were developed in the biostatistics literature to model familial aggregation of age of onset. However, these frailty models can not be used directly for linkage analysis. This paper extends the gamma frailty model by incorporating inheritance vector information and provides a semiparametric approach for linkage testing. For a given inheritance vector at the putative disease locus, we construct an additive genetic gamma frailty for each individual within a nuclear family and use the Cox proportional hazard model to model age of onset. We derive the conditional hazard ratio parameter for sib pairs and define a likelihood ratio based LOD score statistic under our model. The EM algorithm is used for estimating the parameters and the maximum likelihood functions. Simulation study indicated that the proposed method is well behaved and can be more powerful than the non-parametric linkage (NPL) score statistic of GENEHUNTER. The method can be regarded as the variance-component linkage analysis method for age-at-onset data.

Program Nr: 1452 from the 1999 ASHG Annual Meeting

Perl as a Tool for Linkage Analysis. *W. Li[1], F. Haghighi[2]*. 1) Lab Statistical Genetics, Rockefeller Univ, New York, NY; 2) Dept. of Genetics and Development Columbia University, New York, NY.

For an analyst, linkage analysis entails a series of steps involving preparation of input files for specification of both the disease model and pedigree structure, and manipulation of output files to obtain the desired results. These steps are often repetitive, and when accomplished manually can be extremely time consuming and error-prone. We propose to automate such tasks with the aid of Perl scripts. Perl is a very portable programming language that allows for easy manipulation of numbers and text, files and directories and external programs. Besides UNIX, it is availab1e on MS-DOS, Win 95/NT, Macintosh and many other operating systems. Perl is widely used in computer system administration, Web based applications, and bioinformatics. We describe three examples where Perl scripts are used to facilitate linkage analysis: (1) Genome-wide linkage analysis maybe automated given the adoption of a consistent file-name notation. This involves analyzing a large pool of markers covering all 23 chromosomes, where we iteratively traverse all the chromosomes and analyze the marker data using programs from the LINKAGE package or GENEHUNTER. (2) Multiple models are often considered in the effort to find gene(s) underlying complex disorders with unknown etiologies. These models can range in complexity and can include combinations of many factors, such as different levels of disease severity, age and sex dependent penetrances, and environmental effects. For traditional linkage analysis, the input files, namely the parameter file defining the disease model and the pedigree file corresponding to the different liability classes can be quickly generated via a Perl script given a user defined disease model. (3) In assessing a putative linkage result for a marker, it is useful to examine the ranking and magnitude of individual family lod scores. The rankings maybe obtained by parsing the linkage output file and extracting the maximum individual family lod scores by a Perl script. The Perl scripts featured here as well as others are available to the research community at http://linkage.rockefeller.edu/soft/perl.

Program Nr: 1453 from the 1999 ASHG Annual Meeting

Identification of a novel locus for non-syndromic autosomal dominant hearing loss. *X.C. Li[1], S. Angeli[2], T.B. Friedman[3], R.A. Friedman[1]*. 1) CMB, House Ear Institute, Los Angles, CA; 2) Dept. of Otorhinolaryngology, Hospital San Juan de Dios, Fundacion Venezolana de Otologia, Caracas, Venezuela; 3) LMG, NIDCD, NIH, Rockville, MD.

Hearing loss is the most common sensory defect in humans. About one in every 1000 children is affected by severe hearing loss at birth or during early childhood. More than 50% of these cases are due to genetic cause with extensive genetic heterogeneity. A genetic linkage study was conducted on a large Venezuelan family with nonsyndromic autosomal dominant hearing loss. The age of onset of the deafness in this family is in the first decade and there is rapid progression leading to profound hearing loss. The deafness gene segregating in this family is mapped to a 14 cM interval on chromosome 2q, distal to DFNA16. Lod scores >3 were obtained with several markers from this region. This interval harbors PAX3 gene and several cochlear specific ESTs.

Program Nr: 1454 from the 1999 ASHG Annual Meeting

The serotonin transporter gene contributes to the genetic risk of alcoholism. *D. Lichtermann[1], D. Hranilovic[2], P. Franke[1], M. Trixler[3], M. Knapp[4], S.G. Schwab[1], W. Maier[1], D.B. Wildenauer[1].* 1) Department of Psychiatry, University of Bonn, Sigmund-Freud-Str 25, D-53105 Bonn, Germany; 2) Department of Organic Chemistry and Biochemistry, Ruder Boskovic Institute, Zagreb, Croatia; 3) Department of Psychiatry and Medical Psychology, University Medical School of Pecs, Hungary; 4) Institute for Medical Statistics, University of Bonn, Sigmund-Freud-Str 25, D-53105 Bonn, Germany.

Studies in rodents and humans have pointed to a relationship between low brain serotonin turnover and high alcohol intake that may play a crucial role in the initiation and maintenance of alcoholism. Decreased availability of serotonin transporter protein has been found in post-mortem and brain imaging studies of patients with alcoholism. A polymorphism in the promoter of the serotonin transporter gene with differential effect on transcription rate and serotonin uptake function is therefore a prime candidate for an alcoholism susceptibility gene. Initial findings of an association with alcoholism in samples of unrelated cases and controls have, however, failed replication in additional case-control studies and in one less stratification-prone family-based study or at best yielded a trend toward association with the dissocial subtype of alcoholism. This is the first study to convincingly confirm the association in a sample of 92 probands with alcohol dependence (rated by structured interview according to DSM-IV diagnostic criteria) and their parents by Transmission/diseqilibrium test (p=0.0056). The result encourages further studies to explore whether the currently limited benefits of alcoholism treatment with drugs enhancing serotonergic neurotransmission can be increased by targeting it to individuals with this specific genetic vulnerability.

Program Nr: 1455 from the 1999 ASHG Annual Meeting

DNA sequence variation in the fibroblast growth factor receptor (FGFR) genes. *D.L. Lind, D.R. Cox.* Dept. of Genetics, Stanford University, Stanford, CA.

Spontaneous mutations in three of the four FGFR genes have been identified which cause a variety of autosomal dominant diseases. A single disease-causing amino acid change has been found in FGFR1 which causes Pfeiffer Syndrome. 22 disease-causing amino acid changes have been found in FGFR2 which cause Apert Syndrome, Beare-Stevenson Cutis Gyrata, Crouzon Syndrome, Jackson-Weiss Syndrome, and Pfeiffer Syndrome. 16 disease-causing amino acid changes have been found in FGFR3 which cause Achondroplasia, Crouzon Syndrome, Hypochondroplasia, non-syndromic craniosynostosis, Saethre-Chotzen Syndrome, and Thanatophoric Dysplasia Types I and II. To date no disease-causing mutations have been found in FGFR4. The high frequency of disease-causing mutations found in FGFR2 and FGFR3 compared with FGFR1 and FGFR4 suggests a higher mutation rate in these genes. If this is the case, it may be mirrored by increased frequency of variation in the genomic sequence. We are assessing the genomic variation in all four FGFRs by direct sequencing of PCR products from 12 unrelated CEPH Caucasian individuals. Using genomic sequence for FGFR3 and FGFR4 available in Genbank, 1-2 kb PCR products were tiled across the genomic regions covering 16 kb and 13 kb respectively. In addition, we have sequenced a BAC containing the FGFR2 gene, whose genomic region spans 120 kb. 1-2 kb PCR products have been desighed to amplify portions of this genomic region, focusing on the exons and the 2-3 kb adjacent to each exon. A BAC containing FGFR1 has also been identified and is being sequenced. Preliminary data indicate that the amount and frequency of DNA sequence variation in FGFR3 differs from that in FGFR4, in a pattern consistent with an increased mutation rate in FGFR3 versus FGFR4.

Program Nr: 1456 from the 1999 ASHG Annual Meeting

Physical Mapping of the Macular Corneal Dystrophy Locus on Chromosome 16q22. *N. Liu[1,2], S. Dew-Knight[1], G.K. Klintworth[2], J.M. Vance[1]*. 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Department of Ophthalmology, Duke University Medical Center, Durham, NC.

Purpose: Macular corneal dystrophy (MCD) is an autosomal recessive disease with different immunophenotypes. Previously we linked the MCD type I gene to an interval on chromosome 16q22 and suggested that MCD type II locus is linked to the same region. The purpose of the present study was to establish a physical map for the MCD interval using YAC (yeast artificial chromosome) and PAC (P1-derived artificial chromosome) libraries. Methods: The CEPH megabase YAC library on 230 96-well plates was the major source for contig formation. DNAs from these plates were pooled on the basis of a two-dimensional PCR screening strategy. Microsatellite markers within the MCD critical region were screened by PCR to get the positive YAC clones. The PAC library was used to cover gaps that could not be closed with any YACs. Results: A YAC contig was constructed between markers D16S512 and D16S518. A gap within the YAC map was successfully closed with the PAC clones. Conclusion: A physical map covering the entire MCD interval has been established using YAC and PAC libraries. This should significantly facilitate the ultimate cloning of the MCD gene.

Program Nr: 1457 from the 1999 ASHG Annual Meeting

IL13 coding region polymorphism is associated with high total serum IgE level. *X. Liu[1], R. Nickel[2,3], B. Björksten[4], U. Wahn[3], T. Beaty[1], S. Huang[2]*. 1) Department of Epidemiology, Johns Hopkins University, Baltimore, MD, USA; 2) Department of Clinical Immunology, Johns Hopkins University, Baltimore, MD, USA; 3) Department of Pediatrics,Humboldt University, Berlin, Germany; 4) Department of Pediatrics, University Hospital, Linköping, Sweden.

Interleukin-13 (IL13) and interleukin-9 (IL9) are important in the regulation of allergic responses, and their encoding genes are located at chromosome 5q31-33, where both linkage and association studies have provided evidence for existence of susceptibility genes for the total serum IgE level, bronchial hyperreactivity, and asthma. We have tested for the association between IL13 and IL9 coding region polymorphisms and total serum IgE level. This was performed by using extremely high and low IgE level (85th and 15th percentile, respectively, for at least two over four timepoints after gender adjustment) in children of the German Multicenter Atopy Study (MAS-90). The case-control comparisons suggested a significant allelic association between the IL13 coding region polymorphism and high serum total IgE level (38 cases-high IgE and 51 controls-low IgE, odds ratio =2.47, p=0.039), but there is no evidence of association with IL9 polymorphism (odds ratio =0.87, p=0.79). And additional 51 Swedish subjects (26 cases and 25 controls based on the 75th and 25th percentile of the age-gender adjusted IgE distribution) recruited from the International Study of Asthma and Allergies in Childhood (ISAAC) cohort further confirmed the significance of variation at IL13 coding region, with an odds ratio of 4 (p-value=0.025), and no significant association for IL9 polymorphism. These results suggest the IL13 coding region variant maybe associated with the development of high serum IgE phenotype.

Program Nr: 1458 from the 1999 ASHG Annual Meeting

An Extension of the Haseman-Elston Method for Arbitrary Sibships. *Y. Liu[1], D. Tritchler[1,2], S. Bull[1,3]*. 1) Public Health Sciences, University of Toronto, Toronto, Ontario, Canada; 2) Ontario Cancer Institute, University of Toronto, Toronto, Ontario, Canada; 3) Samuel Lunenfeld Research Institute of Mount Sinai Hospital, Toronto, Ontario, Canada.

Haseman & Elston (1972) developed a sib-pair method (HE) for detecting linkage to quantitative trait loci. The method is based on a simple linear regression of the squared sib-pairs trait difference on the proportion of alleles shared identical by descent (IBD) at a marker locus. Linkage is detected by a negative slope which has been traditionally assessed by a standard t-test. For sib-pairs, it has been pointed out that the pair sums also express information about linkage [Elston, 1969; Wright, 1997]. When the size of sibships is greater than two, the sibships were usually analyzed in two ways: using all n-1 independent pairs of phenotyped sibs or using all n(n-1)/2 distinct pairs of phenotyped sibs. The first way loses information and the second ignores the dependency among the sib pairs. In this paper, we introduced a new model-free linkage strategy, called HE+, which is similar to the HE method in terms of the formulation and take into account of the multivariate structure of the sibships. HE+ method can be easily extended to the case of multiple trait loci. The simulated family data from Genetic Analysis Workshop 10 [Goldin et al. 1997] was used to compare HE+ and HE method under various circumstances.

Program Nr: 1459 from the 1999 ASHG Annual Meeting

High resolution mapping of the Blepharophimosis, Ptosis, and Epicanthus inversus Syndrome (BPES) locus. *A. Loi[1], G. Pilia[1], L. Crisponi[1], M. Deiana[1], P. Gasparini[2], L. Bisceglia[2], P. Amati[3], D. Bonneau[3], M. Rocchi[4], S. MacMillan[5], P. Ma[5], E. Chen[5], D. Schlessinger[5], A. Cao[1].* 1) IRTAM, CNR, Cagliari, Italy and NIA, NIH, Bethesda, MD; 2) IRCCS, San Giovanni Rotondo, Italy; 3) CHU, Poitiers, France; 4) Universita' di Bari, Bari, Italy; 5) Perkin Elmer Co, Foster City, CA and Washington University, S.Louis, MO.

Blepharophimosis, Ptosis, and Epicanthus inversus Syndrome (BPES)(MIM110100) is an autosomal dominant disorder with developmental abnormalities affecting craniofacial features and ovarian function. In families with BPES type I the affected females have primary ovarian failure (POF) and therefore do not transmit the condition to children. In contrast, in BPES type II the craniofacial defects are not associated with POF, and both affected males and females are fertile. Linkage studies assigned the BPES locus to 3q22-q23. Aiming at positional cloning of the BPES gene, we have constructed a high-resolution physical map of the region using YAC, BAC, and cosmid clones. The availability of a BPES patient with a balanced de novo translocation t(3;7)(q23-q32) allowed us to map the locus for the disorder within 100 Kb. To facilitate the identification of candidate genes in the region,160 kb of genomic DNA was sequenced in bacterial clones spanning the breakpoint. Complete characterization of the genomic sequence is underway, with candidate genes tested for associated mutations in families segregating BPES.

Program Nr: 1460 from the 1999 ASHG Annual Meeting

Power and Significance levels of tests incorporating the unaffected sibs in families ascertained on the basis of one or more affected offspring. *K.L. Lunetta[1,2], N.M. Laird[2]*. 1) Department of Biostatistical Science, Dana Farber Cancer Institute; 2) Department of Biostatistics, Harvard School of Public Health, Boston, MA.

We derive a score test statistic T_{au} for testing transmission disequilibrium which incorporates the information from unaffected offspring as well as affected offspring, and is equivalent to T_{sib} of Whittaker & Lewis (1998). T_{au} takes a weighted average of the contributions of affected and unaffected offspring. We examine the power and significance levels of T_{au} using several choices of weights, including 1) using affecteds only (the traditional TDT), 2) Whittaker & Lewis weights (weight affecteds by 1- population prevalence) and 3) optimizing the statistic over the weight, and then using the correction suggested by Davies (1977) to adjust the p-value.

To compare power, we simulate samples of 100 nuclear families, each with two offspring. The families are ascertained into the sample if at least one offspring is affected; the proportion of families with the second offspring affected depends on the sibling risk ratio of the genetic model. We use a simple genetic model in which we are testing the actual disease allele, and specify allele frequency and penetrances.

In summary, we find that transmission information from the unaffected siblings of cases can in some cases add power when testing transmission disequilibrium, particularly if disease prevalence is high. If the population prevalence is used as the weight, including unaffected sibs will not decrease the power of a test. When accurate estimates of population prevalence are not available for common traits, optimizing the test statistic over the weight and applying Davies (1977) correction is often more powerful than using affected offspring alone. Future work will compare power for genetic models which are more appropriate for complex disease, incorporating a shared genetic and environmental risk for sibs beyond the gene allele being tested.

Program Nr: 1461 from the 1999 ASHG Annual Meeting

Candidate gene analysis in the lupus-prone mouse strain, BXSB. *M.A. Maibaum[1], G. Fu[1], M.E.K. Haywood[1], S. Uribe[1], S.J. Rose[1], R.B. Sim[2], M.J. Walport[1], B.J. Morley[1]*. 1) Rheumatology Section, Imperial College, Hammersmith, London, U.K; 2) MRC Immunochemistry Unit, Oxford University, Oxford, U.K.

We have performed a genome-wide linkage analysis for disease susceptibility genes in the lupus-prone recombinant inbred mouse strain, BXSB. We have analyzed reciprocal backcrosses between the non-autoimmune strain C57BL/10 (B10) and BXSB. A number of intervals showed strong linkage to disease susceptibility including four intervals on chromosome 1 (*Bxs1-4*) and one on chromosome 3 (*Bxs5*). We have initiated a candidate gene screen in these areas by comparing DNA sequences between the B10 strain and BXSB and by additional comparison with other lupus-prone strains (MRL, NZB, NZW).

Cd152 and *Cd28*, located within interval *Bxs1*, showed no variation between BXSB and B10 in the coding sequence. For *Fcgr2*, a strong candidate gene in the *Bxs3* interval, we have previously identified coding sequence polymorphisms. We have now identified sequence variation within the first 200bp of the RNA polymerase start site, which may lie in promoter control elements. Linkage analysis performed using the variation between B10 and BXSB promoter sequences as a marker, indicated that this region was closely associated with anti-nuclear antibody production and splenomegaly. The functional correlates of these sequence variants are under investigation.

Factor I, a complement system control protein, maps within the *Bxs5* interval on chromosome 3. We identified 16 nucleotide differences between BXSB and B10 *Cfi*, 11 of which alter amino acids. Preliminary analysis demonstrated that BXSB sera has higher factor I C3b-cleavage activity than B10 sera. We are presently purifying factor I from BXSB and B10 sera to assess this potential functional difference further.

Program Nr: 1462 from the 1999 ASHG Annual Meeting

Functional studies of mammalian FMRP in yeast. *D.M Absher[1], S.T. Warren[2]*. 1) Dept. of Biochemistry, Emory University School of Medicine, Atlanta, GA; 2) Howard Hughes Medical Institute, and Depts. of Biochemistry, Genetics, and Pediatrics, Emory University School of Medicine, Atlanta, GA.

Fragile X syndrome is a result of the loss of FMRP, the protein product of the FMR1 gene. This is generally caused by an expansion of the CGG repeat in the 5' UTR leading to transcriptional silencing. FMRP is known to be an RNA binding protein, that selectively binds a subset of mRNAs and interacts with polyribosomes as a large mRNP complex. A severely affected patient has been described with an I304N missense mutation which leads to an mRNP of reduced mass which fails to interact with ribosomes. Such data suggests that FMRP may play a role in the translation of certain messages, although its function remains unknown. To better understand the function of FMRP and its role in translation, we have developed a model system in which mammalian FMRP is expressed in *Saccharomyces cerevisiae*, a species without an obvious FMRP homologue. We have expressed FMRP from an integrated plasmid targeted to the Leu2 locus, using the ADH1 promoter. Sucrose gradient analysis has shown that FMRP can associate with yeast polyribosomes and that the I304N mutation can disrupt this association. Furthermore, EDTA-resistant mRNP complexes are detected in these cells, and the I304N mutant complex is abnormally small, just as it is in human cells. This suggests that FMRP engages in nucleoprotein interactions required for association with ribosomes and that at least some of the partners are conserved in yeast. Indeed, FMRP coimmunoprecipitates with at least two yeast proteins which are currently being identified by mass spectrometry. Given the ease of analysis and substantial characterization of translation in yeast, this model system may provide an approach for the functional dissection of FMRP.

Program Nr: 1463 from the 1999 ASHG Annual Meeting

Modelling Familial Hypertrophic Cardiomyopathy Using YAC Transgenesis. *S. Al-Mahdawi[1], S. Chamberlain[1], C. Huxley[1], Z. Webster[2], D. Nunez[3].* 1) Molecular Genetics, Imperial College of Science, Technology and Medicine, London, United Kingdom; 2) MRC Clinical Sciences Centre, Hammersmith Hospital, Imperial College of Science, Technology and Medicine, London, United Kingdom; 3) Clinical Pharmacology, Hammersmith Hospital, Imperial College of Science, Technology and Medicine, London, United Kingdom.

Hypertrophic cardiomyopathy is characterised by hypertrophy of the left ventricle of the heart with myocyte and myofibrillar disarray. Mutations in the coding sequences of several sarcomeric proteins, including alpha-tropomyosin, have been implicated in the pathology of this disorder. To facilitate an understanding of the natural progression of the hypertrophy, we have generated transgenic mice carrying the Asp175Asn mutation. To mimic tissue-specific and physiological expression of the wild-type protein, the mutation has been introduced in the context of the alpha-tropomyosin gene locus contained within a 210 kb human yeast artificial chromosome clone, which should include the endogenous regulatory elements. Three founder animals carrying the mutated full-length gene have been identified to date, with transmission of the YAC transgene to the expected 50% of offspring in each case. Western blot analysis using a human specific antibody demonstrated expression of the human transgene in skeletal muscle. The pathophysiological consequences of the mutation are currently being investigated at the molecular and histopathological levels.

Program Nr: 1464 from the 1999 ASHG Annual Meeting

COMT is not associated with Obsessive Compulsive Disorder - family-based vs. population-based designs. *J.P. Alsobrook[1], A.H. Zohar[2], M. Leboyer[3], N. Chabane[4], R.P. Ebstein[5], D.L. Pauls[1]*. 1) Child Study Center, Yale Univ. Sch. Medicine, New Haven, CT; 2) Scheinfeld Center for Human Genetics in the Social Sciences, Department of Psychology, Hebrew University of Jerusalem, Israel; 3) Service de Psychiatrie d'adultes, Hôpital Henri Mondor et Albert Chenevier, AP-HP, Creteil, France; 4) Sevice de Psychopathologie de l'enfant et de l'adolescent, Hôpital Robert Debre, Paris, France; 5) Research Laboratory, S. Herzog Memorial Hospital, Jerusalem, Israel.

A polymorphism in the coding region of the catechol-O-methyltransferase gene (COMT) was previously reported to be associated with Obsessive Compulsive Disorder (OCD), particularly in male probands[1]. That report was based on a case-control paradigm that examined OCD probands and an unrelated control sample matched for ethnicity. Because molecular genetic case-control studies can exhibit Type I error in the face of population stratification of alleles[2], we attempted to replicate the previous finding using a family-based design in a haplotype relative risk analysis (HRR)[3]. Fifty-six OCD probands and their parents were genotyped for the COMT locus using established methods[4]. Analysis of allele and genotype frequencies between the proband genotypes and the control (parental non-transmitted) genotypes gave no evidence of association. A further analysis of gender and COMT alleles in OCD probands also gave no evidence of association. These findings indicate that 1) COMT is probably not associated with OCD; and 2) caution is necessary in the design and interpretation of molecular genetic association studies that use a case-control paradigm.

[1]PNAS 94:4572-2575 (1997).

[2]Am J Hum Gen 57:455-464 (1995).

[3]Hum Hered 42:337-346 (1992).

[4]Am J Med Gen 67:468-472 (1996).

Program Nr: 1465 from the 1999 ASHG Annual Meeting

Genetic prediction of antipsychotic response. *M.J. Arranz[1], J. Munro[1], J. Birkett[1], A. Bolonna[1], D. Mancama[1], M. Sodhi[1], K.P. Lesch[2], J.F.W. Meyer[2], P. Sham[1], D.A. Collier[1], R.M. Murray[1], R.W. Kerwin[1]*. 1) Dept Psychological Med, Inst Psychiatry, London, England; 2) Department of Psychiatry, University of Wuerzburg, Germany.

Prediction of clinical response to pharmacological treatments through pharmacogenomic analysis of selected targets may lead to the direct selection of the most beneficial treatment for each individual. In previous studies we hypothesised that response to antipsychotics may be influenced by mutations in the targeted neurotransmitter receptors. In support of this hypothesis, we found associations between polymorphisms in the serotonin 2A (5-HT2A) and 2C (5-HT2C) receptor genes and response to the potent antipsychotic clozapine. However, these polymorphisms could not fully explain the individual's variation in response to clozapine treatment. We have now investigated 19 genetic variants in neurotransmitter receptors targeted by clozapine and in the serotonin transporter (5-HTT) in a sample of 200 schizophrenic patients treated with the drug. A combination of 6 of the polymorphisms studied in 4 different loci (5-HT2A, 5-HT2C, 5-HTT and Histamine type 2) results in 76.7% success in the prediction of clozapine response (p= 0.0001). These results constitute the first report of the use of pharmacogenetics for the individualisation of psychiatric treatment and will form the basis for a simple test to enhance the usefulness of clozapine.

Program Nr: 1466 from the 1999 ASHG Annual Meeting

The role of protein misfolding in the neurodegenerative disease, Spinal and bulbar muscular atrophy. *C.K. Bailey[1,2], D.E. Merry[2]*. 1) Univ. of Pennsylvania, Neuroscience Graduate Group, Philadelphia, PA; 2) Thomas Jefferson Univ., Dept. of Biochemistry and Molecular Pharmacology, Philadelphia, PA.

Spinal and bulbar muscular atrophy (SBMA) is a neurodegenerative disease caused by expansion of a polyglutamine repeat within the androgen receptor (AR). A common feature of polyglutamine repeat diseases is protein misfolding, as evidenced by the presence of ubiquitinated neuronal intranuclear inclusions, containing the mutated protein. Analysis of such inclusions in motor neurons of SBMA patients reveals the presence of only amino-terminal AR epitopes, suggesting that the amino terminus of expanded repeat AR is pathogenic. Consistent with this, we have shown that an amino-terminal portion of the expanded repeat AR is more toxic to Cos cells than full length AR. Using this *in vitro* system, we have shown altered metabolism for expanded repeat AR, including aggregation and proteolytic processing in a repeat length-dependent manner. Our finding of co-localization of the molecular chaperones, Hsp70 and Hsp40, with AR inclusions supports the idea of protein misfolding in this disease.

Molecular chaperones are known to bind to misfolded proteins in order to refold the proteins and to prevent aggregation. Since co-chaperones of the Hsp40 family may be present in cells in limited amounts, we overexpressed an Hsp40 family member, HDJ-2/HSDJ, in our *in vitro* system to study the effects on AR protein metabolism. Co-expression of HDJ-2/HSDJ with truncated, expanded repeat AR resulted in a significant, although modest, decrease in aggregate formation, in both Cos and HEK293T cells. HDJ-2/HSDJ J domain mutants showed a similar decrease in aggregation, indicating that this effect is not J domain dependent. We are currently investigating the effect of HDJ-2/HSDJ over-expression on the cellular toxicity caused by expanded repeat AR.

These studies suggest that abnormally folded expanded polyglutamine repeat AR may overwhelm the cell's molecular chaperone systems, thus leading to aggregation. Our current studies will allow us to determine if the process of protein misfolding contributes to cell death.

Program Nr: 1467 from the 1999 ASHG Annual Meeting

Increased Prevelance of Alzheimer Disease in an Arab-Israeli Community. *C.T. Baldwin[1], A. Bowirrat[4], A. Korczyn[4], C. Adams[1], L. Farrer[2], R. Friedland[3]*. 1) Ctr Human Genetics, Boston Univ Sch Medicine, Boston, MA; 2) Genetics Program, Dept of Medicine, Boston Univ Sch Medicine, Boston, MA; 3) Department of Neurology, Case Western Reserve Univ, Cleaveland, OH; 4) Department of Neurology, University of Tel-Aviv, Israel.

In a population-based study of Alzheimer disease (AD), we screened all elderly residents (855 individuals) of an Arab community in northern Israel. Fourteen families (hamulas) founded this community several hundred years ago and consanguinity is commonly practiced. In those over the age of 60, we observed an unusually high prevalence of AD (20.5% of those > 60; 60.5% of those > 85). This prevalence is 2-4 fold higher than reported for any other population, even after adjustment for age, education and gender. The frequency of APOE e4 alleles was low in this population, 3.5% for non-demented elderly controls and 4.5% for AD cases. Thus, the increased frequency of AD is not due to APOE e4. The occurrence of AD in this community appears to be clustered in families; more than one-third of the 168 prevalent cases occur in one hamula (#13). We hypothesize that this is may be due to a founder effect in this genetic isolate as well as a result of consanguinity. A 10 cM genome wide scan was conducted in 5 cases and 5 non-demented control samples from hamula #13. We expect that an AD predisposing gene would be identical by descent in affected members of this family and excessive allele sharing, or homozygosity among affected individuals would be found. Five chromosomal regions were identified that show a significant association with AD (Fishers Exact $p < 0.05$). One chromosomal location near the presenilin-2 gene on chromosome 1 and the second near a potential locus reported by Kehoe et al., 1999. In summary, the study of AD in this unique genetic isolate has the potential to identify new AD genes, particularly those that are inherited in a recessive fashion.

Program Nr: 1468 from the 1999 ASHG Annual Meeting

Multiple Functional Variations In COL6A1 Coding Region Modulate Risk of Congenital Heart Defects in Down Syndrome. *M.J. Baptista[1], U.L. Fairbrother[1], G.E. Davies[1], D. Trikka[2], T. Davis[1], C. Baldock[3], C.M. Kielty[3], A.M. Kessling[1]*. 1) Medical and Community Genetics, Imperial College, , Harrow, Middlesex, England; 2) Department of Human and Molecular Genetics, Baylor College of Medicine, Houston, Texas; 3) School of Biological Sciences, Manchester University, 2.02 Stopford Building, Oxford Road, Manchester, M13 9PT.

Down syndrome (DS) is associated with a much higher incidence of congenital heart defects (CHD) than is seen for the general population (40% compared to 0.8% euploid individuals). The COL6A1-A2 locus on chromosome 21q22.3 is included in the critical region for CHD associated with DS. Variation in the COL6A1 region is associated with CHD.

We have used SSCP and direct sequencing methods to analyse each of the globular regions encoding exons of COL6A1. To date we have found twelve novel polymorphisms and functional variations in the 3' end of the coding sequence. The distribution of these variants has been studied in 97 families having a child with DS with or without a heart defect.

Individuals with DS not having mostly wild type haplotypes found in COL6A1 for the SSCP or the RFLP data confers a relative risk of 5.1 and an odds ration of 11.25 (p=0.008 Fishers exact test) for having a CHD.

In 33 UK/Irish families there are eleven unequivocal haplotypes and eight probable unique haplotypes, many seen in only a single individual. All of these haplotypes appear to be functional in the diploid state since they exist in euploid parents who do not have CHDs.

We propose a disease mechanism whereby the expression of more than two haplotypes disrupts the stoichiometry of the collagen VI molecule. This may lead to the modification of the tertiary structure of the collagen VI "beaded filaments." The resulting disturbance in function of collagen VI (relative to the euploid state) would lead to the failure of the endocardial cushions to fuse, resulting in a CHD.

Program Nr: 1469 from the 1999 ASHG Annual Meeting

Assessment of the methylenetetrahydrofolate reductase (MTHFR) C677T genotype and neural tube defect risk in a south Texas Hispanic population. *R.C. Barber[1], B.S. Joggerst[2], S.L. Shalat[2], R.H. Finnell[1]*. 1) Center for Molecular Genetics, University of Nebraska Medical Center, Omaha, NE; 2) Environmental and Occupational Health Sciences Institute, UMDNJ, Rutgers, Piscataway, NJ.

Intracellular methionine and homocysteine metabolism are controlled primarily by the folate-dependent enzyme, 5,10-methylenetetrahydrofolate reductase (MTHFR). Although methionine synthase catalyzes the final reaction, MTHFR is responsible for the rate limiting step in the cycle that converts homocysteine to methionine. A mutant MTHFR allele (C677T) that encodes a thermolabile variant of the protein with reduced activity, has been linked to elevated serum homocysteine levels in TT homozygous individuals. Although studies of Dutch and Irish populations have reported an association between homozygosity for this mutation and elevated neural tube defect (NTD) risk, other studies have either found no, or a much smaller association. The MTHFR C677T genotype was determined in a case-control sample collected from El Paso, Webb and Hidalgo counties in the Rio Grande valley of Texas. This population was specifically investigated because NTD prevalence is known to be generally greater among Hispanic than non-Hispanic Caucasians, and because a cluster of NTDs was detected in this region in 1991, with increased NTD rates being observed again in 1998. Crude odds ratios and 95% confidence intervals were computed using SAS, and exact probabilities were calculated using Fisher's Exact Test. Allele frequencies in different populations were compared by Chi-square. Although no significant association was observed between any genotype and NTD risk, genotype frequencies in the overall sample population were significantly different from those reported for Irish or Hispanic populations. In addition, the frequency of the T allele (0.4892) was higher in this study than has been previously reported. The observations presented here as well as conflicting reports from the literature illustrate the complexity of the relationship between the MTHFR C677T polymorphism and NTD risk. Accordingly, the impact of this polymorphism on NTD etiology is far from being resolved. Funding for this study was provided by NIH grant ES06650.

Program Nr: 1470 from the 1999 ASHG Annual Meeting

Positive association of nonsyndromic cleft palate only (CPO) and aryl hydrocarbon receptor nuclear translocator 2 (*ARNT2*). . *L.L. Barrow[1], M.E. Wines[2], P.A. Romitti[3], B.C. Holdener[2], J.C. Murray[3]*. 1) Otolaryngology, Univ. of Iowa, Iowa City, IA; 2) Biochemistry and Cell Biology, State Univ. of NY at Stony Brook, Stony Brook, NY; 3) Pediatrics, Univ. of Iowa, Iowa City, IA.

Nonsyndromic orofacial clefts have an incidence of 1/1000 live births. Population genetic and embryologic studies suggest that cleft palate only (CPO) may be a distinct clinical entity from cleft lip with or without cleft palate (CL/P). Clefts are thought to be multifactorial in etiology, with genetic, environmental, and developmental determinants all playing a role. Mice are valuable model systems to identify potential risk factors. The *c112k* mouse line demonstrates *ARNT2* deletion and a complex phenotype including CPO and thymic hypoplasia. *ARNT2* localizes to a conserved linkage group on mouse Chr 7 that is syntenic with human Chr 15q23-25. This chromosomal region was previously identified as a teratogen-induced clefting susceptibility locus in a genome-wide scan of AXB and BXA recombinant inbred mice. To determine whether *ARNT2* influences human craniofacial development, we identified the human *ARNT2* gene and conducted genomic structural analysis. Mutational screening was performed in infants with nonsyndromic CPO or CL/P identified by the Iowa Birth Defects Registry, an active population-based surveillance system. No obvious disease-causing mutations were detected by SSCP analysis. A microsatellite marker, *GATA89D04*, was identified within an intron of human *ARNT2*, and linkage disequilibrium of nonsyndromic CPO and CL/P parent-infant trios was conducted, following transmission of the 244 bp allele. AFBAC analysis (CPO=20; CL/P=37) demonstrated a positive association with CPO (c^2=11.90; $p<0.02$) but no association with CL/P (c^2=1.94; $p>0.05$). TDT analysis (CPO=28; CL/P=48) confirmed a positive association with CPO (c^2=6.26; $p<0.05$) but no association with CL/P (c^2=0.69; $p>0.05$). Future analyses include evaluation of further CPO parent-infant trios and assessment of environmental exposures. Of particular interest, the teratogen 2,3,7,8-tetrachlorodibenzo-p-dioxin acts through the aryl hydrocarbon receptor pathway to produce dose-dependent CPO and thymic wasting in mice exposed *in utero*.

Program Nr: 1471 from the 1999 ASHG Annual Meeting

Autistic Disorder and chromosome 7: Analysis of an inversion breakpoint in a multiplex family. *S. Basu[1], A. Ashley-Koch[1], C.M. Wolpert[1], M.M. Menold[1], N. Matsumoto[2], C.M. Powell[3], M.B. Qumsiyeh[1], M.L. Cuccaro[4], D.H. Ledbetter[2], E.D. Green[5], J.M. Vance[1], M.A. Pericak-Vance[1], J.R. Gilbert[1]*. 1) Department of Medicine, Duke University Medical Center, Durham, NC; 2) Department of Human Genetics, University of Chicago, Chicago, IL; 3) Department of Pediatrics, University of North Carolina, Chapel Hill, NC; 4) WS Hall Psychiatric Institute, University of South Carolina, Columbia, SC; 5) NHGRI, NIH, Bethesda, MD.

Autistic Disorder (AD) is a developmental affliction characterized both by severe impairments in reciprocal social interaction and communication, as well as by stereotyped patterns of behavior. Genomic screen results from several groups (IMGSAC, 1998; Bass et al., 1998; Philippe et al., 1999) indicate the presence of an AD susceptibility gene on the long arm of chromosome 7, in the region of 7q31-q35. Cytogenetic abnormalities associated with an autistic phenotype found to overlap the region of positive linkage have previously been reported (de la Barra et al., 1986; Sarda et al., 1988; Lopreiato et al., 1992) and can help to narrow the region of interest. We have identified a family (Duke 7543) with multiple affected members segregating for a paracentric inversion, inv(7)(q22q31.2), which overlaps our region of suggested linkage on chromosome 7 (Ashley-Koch et al., ASHG 1999). We postulate that the inversion is disrupting expression of the putative AD gene on 7q; we are therefore isolating and characterizing the inversion breakpoints. Using a series of 12 BAC clones for FISH analysis, we have narrowed the 7q31.2 breakpoint region to a 350 kb interval several centimorgans proximal to CFTR, and are in the process of constructing a physical map of the area. Pulse field mapping of the 350 kb region is in progress to identify CpG islands, indicative of candidate genes. In addition, we are constructing a cosmid library from an affected member of our pedigree to physically fine map and characterize both inversion breaks. Physical and gene mapping data, as well as a genomic contig of the candidate region, will be presented.

Program Nr: 1472 from the 1999 ASHG Annual Meeting

Quantitative fluorescence PCR (QF-PCR) of chromosome 11p15.5 markers in Beckwith-Wiedemann syndrome (BWS): Detection of microduplications and microsegmental uniparental disomy (UPD). *L.S. Beischel[1], M.M. Haag[1], D.H. Viskochil[2], S.M. Phillips[1], J.P. Johnson[1]*. 1) Medical Genetics, Shodair Hospital, Helena, MT; 2) Pediatrics/Genetics, U. of Utah, Salt Lake City, UT.

BWS, a childhood disorder of tissue overgrowth and cancer susceptibility, is associated with abnormalities of expression of imprinted genes at 11p15.5. Increased dosage of growth-promoting genes expressed from the chromosome of paternal origin, or decreased dosage of growth-suppressing genes expressed from the chromosome of maternal origin, or both, may cause this syndrome. About 1% of patients have visible chromosome rearrangements (translocation, inversions and duplications), 15% have paternal uniparental disomy (UPD, usually segmental and mosaic), and 20% are familial, with maternal inheritance. More than half of BWS patients lack a characterized genetic cause for their condition. We looked for paternal UPD using QF-PCR for 10 polymorphic DNA markers from 11p15.5 in five families with an affected child. In two patients, we found probable de-novo DNA duplications of paternal origin within the critical region of chromosome 11, predicting functional disomy for paternally expressed genes. The duplicated alleles had twice the peak areas of the single copy alleles. Mosaic segmental paternal UPD, while possible, is unlikely given identical 2:1 allele ratios in both patients. A third patient has a very small region lacking maternal alleles, probably due to microsegmental paternal UPD, again predicting disomy for paternally expressed genes. These anomalies were not visible by G-banded cytogenetic analysis of patients 1 and 3 (study pending on patient 2). We hypothesize that such microduplications and microsegmental loss of maternal alleles (probable paternal UPD) are more frequent than visible rearrangements of chromosome 11p, and have previously been undetected by cytogenetic and molecular techniques. QF-PCR, using polymorphic markers localized to the region of imprinted genes at 11p15.5, can potentially detect aberrations of gene copy number or parental origin at higher resolution than previously available. Results may be helpful in genetic counseling and in defining a molecular etiology of BWS.

Program Nr: 1473 from the 1999 ASHG Annual Meeting

Interleukin-1-induced Fas is not always nitric oxide dependent in mouse islet cells. *S. Bertera, A. Alexander, M. Crawford, M. Trucco.* Division of Immunogenetics, Department of Pediatrics, University of Pittsburgh School of Medicine, Rangos Research Center, Children's Hospital of Pittsburgh, Pittsburgh, PA.

Insulin dependent diabetes mellitus (IDDM) is an autoimmune disease characterized by the destruction of the insulin-producing beta cells of the islets of Langerhans in the pancreas. To understand the underlying basis of Type 1 diabetes pathogenesis, attention must be focused on the factors that permit or provoke this autoimmune attack. Interleukin-1 beta (IL-1b), a proinflammatory cytokine, is the major secreted form of IL-1 and related to tumor necrosis factor alpha (TNF-a). IL-1b has recently been shown to upregulate Fas on the surface of the insulin producing beta cells in the pancreas, priming them for destruction by the Fas (CD95/APO-1) dependent apoptotic cell death pathway. Nitric oxide (NO), which is derived from L-arginine, is thought by many to play an important role in the apoptotic cell death of pancreatic islets, resulting in IDDM. The NO radical, when present in moderate amounts, can function as a cellular signaling molecule or regulator of gene expression. During the insulitis process in humans, it has been reported that secretion of IL-1 by activated macrophages induces transcription and translation of the inducible nitric oxide synthase (iNOS) gene and produces NO in abundance. It was then hypothesized that the Fas mediated apoptosis pathway of human pancreatic islets is upregulated in response to NO production by the iNOS gene. However, we have evidence that the same may not be true for rodent beta cells. Our studies show, using islets from an iNOS knockout mouse model, through *in vitro* NO suppression with arginine analogs and arginine-free media, and flow cytometric analysis, that Fas can be upregulated on the surface of mouse islet cells in response to IL-1b without the presence of L-arginine, the iNOS gene or NO, suggesting that in mice the Fas mediated apoptotic cell death pathway is not necessarily nitric oxide dependent.

Program Nr: 1474 from the 1999 ASHG Annual Meeting

Investigation of polymorphisms in the NMDAR1 gene in association with schizophrenia. *A.A. BOLONNA, J. Munro, M.J. Arranz, A.J. Makoff, R.W. Kerwin.* psychological medicine, INSTITUTE OF PSYCHIATRY, LONDON, ENGLAND.

The glutamate system, mediating the majority of excitatory transmission, has been implicated in the complex aetiology of schizophrenia. An initial report of decreased glutamate concentrations in the cerebrospinal fluid of schizophrenic patients (Kim et al., 1980) was supported by observations that phencyclidine (PCP), a non-competitive NMDA antagonist, induces a psychotomimetic effect in normal subjects closely resembling that of schizophrenia (Domino and Luby, 1981). Additional suggestive evidence include alterations in receptor binding and messenger RNA expression levels of glutamate receptor subunits in the post-mortem brain of patients (Kerwin et al., 1990; Harrison et al., 1991). Therefore, the genes encoding the glutamate receptor family are candidates in the aetiology of the disorder. We have investigated whether variations in the NMDAR1 gene, the key subunit of the NMDA receptor, influences the genetic predisposition to schizophrenia.The NMDAR1 gene, 22 exons in length, was systematically screened for mutations, using single strand conformation polymorphic (SSCP) analysis, in 50 cases and an equal number of controls. The sample population included patients of British Caucasian origin (DSMIII/IVR classification) and ethnically matched controls.Two silent polymorphisms were identified in the coding region of the gene (exon 6-A/G and exon 7-A/G) and an additional five in non-coding regions (-855-G/C, Int10-G/A, Int11-A/G, Int12-del7 and Int14-C/T). The frequent polymorphisms were genotyped in cases and controls, but association was not detected with schizophrenia. However, haplotype analysis revealed that the polymorphisms were in strong linkage disequilibrium ($p<0.00001$) and particular haplotype combinations showed an association with schizophrenia ($p=0.01$).In conclusion, analysis of variations in the NMDAR1 gene in association with schizophrenia does not suggest a major role for this gene in conferring susceptibility to the disorder.

Program Nr: 1475 from the 1999 ASHG Annual Meeting

CFTR gene mutations in sarcoidosis patients. *C. Bombieri[1], F. Belpinati[1], M. Luisetti[2], L. Casali[2], P.F. Pignatti[1].* 1) Section of Biology and Genetics, DMIBG, Univ. Verona, Italy; 2) Inst. Respir. Dis., IRCCS, S. Matteo Hospital, Univ. Pavia, Italy.

Sarcoidosis is a multisystem immuno-mediated granulomatous disorder with unknown aetiology. The diagnosis of sarcoidosis depends on finding noncaseating granulomas in affected tissue, therefore infectious agents known to cause granulomatous inflammation have been investigated as potential aetiological agents. Ethnic variation in incidence and familial clustering of the disease suggest an inherited susceptibility. The relative risk in siblings patients is supposed to be approximately 2.0. Studies performed to determine risk factors remain inconclusive. During a CFTR gene mutation screening in pulmonary diseases, we found that 5/8 (62%) sarcoidosis patients had a mutations, as a serendipity finding (Hum. Genet. 103:718-22, 1998). This high incidence suggested us to analyse a second series of patients to confirm the data. 26 new patients and 65 control individuals were recruited. 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 19/26 patients and for all the controls. In 6/19 patients at least one mutation was found (31%). This increase is not statistically different from the mutations found in controls. The total number of CFTR mutation carriers in completely screened patients is 11/27 (41%), a significant increase over controls (11/65; Fisher's exact test p=0.017). One of the patients presented 3 mutations: R75Q, 186-13C/G, and 1898+3A/G (phase unknown). Moreover, 6 samesense mutations were found in patients, not statistically different from controls (9/65). In the remaining 7 patients, 18/27 exons have been screened: two mutations (exon 16, to be sequenced, and L997F), were found in 2 patients. In conclusion, the data obtained in an enlarged series of sarcoidosis patients shows an excess of CFTR gene mutations, which may indicate an involvement of the gene in disease etiopathogenesis and/or progression.

Program Nr: 1476 from the 1999 ASHG Annual Meeting

Association study of the dopa decarboxylase gene in bipolar affective disorder and schizophrenia. *A.D. Borglum[1], T.G. Bruun[2], M. Hampson[4], T.E. Kjeldsen[1], P. Lodge[4], H. Ewald[2], O. Mors[2], G. Kirov[3], W. Muir[4], V. Murray[4], C. Russ[3], B. Freeman[3], D. Blackwood[4], D.A. Collier[3], T.A. Kruse[5].* 1) Dept Human Genetics, Aarhus Univ, Aarhus, Denmark; 2) Dept Psychiatric Demography, Psychiatric Hospital in Aarhus, Denmark; 3) Institute of Psychiatry, London, United Kingdom; 4) Dept Psychiatry, Royal Edinburgh Hospital, Edinburgh, Scotland; 5) Dept Clinical Biochemistry and Genetics, Odense University Hospital, Denmark.

Dopa decarboxylase (DDC) is a regulated enzyme required in the synthesis of the monoaminergic neurotransmitters dopamine, noradrenalin, adrenalin, and serotonin. In addition, DDC is rate limiting in the production of trace amines such as 2-phenylethylamine (2-PE) which have neuromodulating capabilities. DDC is regarded as a candidate gene for predisposition to a variety of neuropsychiatric disorders including bipolar affective disorder (BPAD) and schizophrenia.

We identified two novel variants in the DDC gene: a 1-bp deletion in the neuronal promoter and a 4-bp deletion in the neuronal exon 1. Both deletions affect putative binding sites for known transcription factors, suggesting a possible functional impact at the level of expression. The two variants were applied in association studies of BPAD and schizophrenia. The BPAD study investigated 80 Danish and 112 English patients, and 223 Danish and 349 English controls. The schizophrenia study comprised 174 patients and 200 controls from Scotland. A significant association was found between the 1-bp deletion and BPAD with P-values of 0.037 (allelic) and 0.021 (genotypic). The frequency of the 1-bp deletion was 13.3% in patients and 9.4% in controls with a corresponding odds ratio of 1.48 (95% CI: 1.02-2.15). A replication study of this finding is currently in progress, analyzing 150 BPAD patients and 200 controls from Scotland.

No significant association was found between the DDC variants and schizophrenia.

The results presented suggest that DDC may act as a minor susceptibility gene for BPAD, whereas no support was found for the possible involvement of DDC in schizophrenia.

Program Nr: 1477 from the 1999 ASHG Annual Meeting

Systematic mutational analysis of the human metabotropic glutamate receptor 3 (mGluR3): association analysis in patients with schizophrenia and bipolar affecitve disorder. *S. Bort[1], S. Cichon[1], T. Schulze[2], D.J. Müller[2], M. Albus[3], E. Franzek[4], M. Knapp[5], M. Rietschel[2], P. Propping[1], M.M. Nöthen[1].* 1) Institute of Human Genetics, University of Bonn, Bonn, Germany; 2) Department of Psychiatry, University of Bonn; 3) Mental State Hospital, Haar; 4) Department of Psychiatry, University of Würzburg; 5) Institute of Medical Statistics, University of Bonn.

Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system. Its actions are mediated by glutamate receptors which fall into ionotropic (iGluR) and metabotropic (mGluR) receptors. The ionotropic receptors are further subdivided into three groups: AMPA, kainate and NMDA receptor channels. The metabotropic receptors are coupled to G-proteins, and regulate the production of intracellular messengers. Several observations implicate brain glutamatergic abnormalities in the pathophysiology of schizophrenia. This evidence includes both human neurochemical and clinical pharmacologic data. In the present study, we have sought to identify mutations in the human metabotropic glutamate receptor 3 (mGluR3) gene by screening genomic DNA samples from 92 unrelated individuals (46 schizophrenic patients and 46 individuals with bipolar affective disorder) by means of single-strand conformation analysis (SSCA). We detected two rare silent sequence variants, namely 627C/T and 873C/T and a common silent variant, 1872C/T. The occurrence of the 1872C/T variant was studied in a large sample of bipolar and schizophrenic patients (n=283 and n=265, respectively) as well as controls (n= 227). We found a significant (P=0.0022) overrepresentation of the 1872T variant in schizophrenic patients when compared to controls. To test for the significance of this finding, we are currently studying the 1872C/T variant in an extended sample of schizophrenic patients and controls as well as in a schizophrenic trio sample using the transmission/disequilibrium test (TDT).

Program Nr: 1478 from the 1999 ASHG Annual Meeting

Skewed X-inactivation associated with abnormal methylation in ICF patients. *D. Bourc'his[1], M. Jeanpierre[2], E. Viegas-Pequignot[1]*. 1) INSERM U383, Hôpital Necker; 2) INSERM U129, CHU Cochin, Paris, FRANCE.

ICF syndrome is a rare autosomal recessive disorder characterized by variable Immunodeficiency, Centromeric instability and Facial dysmorphism. Only one chromosomal region (20q11-q13) has been identified for this pathology despite its heterogeneity. We initially showed that DNA undermethylation is a constitutional feature of ICF genomes and is associated with chromosomal rearrangements typical of the syndrome. The inactive X chromosome of female ICF patients exhibits also a global undermethylated profile which is variable among patients at the molecular level. Intronic CpG sites are systematically demethylated, but 5' CpG islands demethylation of X-repressed genes occurs only in half of the patients. Fonctionnally, the methylation defect does not disturb the major features of the inactivation process which is initiated and maintained in these patients.

We observed that the X chromosome undermethylation is related to a skewed pattern of inactivation. Using the HUMARA assay, skewing was over 90% in the class of ICF patients showing methylated CpG islands. In the second class of patients (demethylated CpG islands), we confirmed this result by the analysis of a XIST expressed polymorphism. The X inactivation skewing was not restricted to leukocytes, thus excluding a monoclonal proliferation of hematopoietic precursor cells or the implication of a specific tissular factor leading to this distorsion. As X chromosome rearrangements or deleterious mutations were not observed in these patients, the skewed X-inactivation is not the result of a secondary cellular selection by relative growth advantages, as usually described in families prediposed to a X inactivation bias. The skewed X inactivation of ICF patients appears as a primary defect in the chromosome choice at the onset of inactivation. The implication of an autosomal factor in this control is suggested by the cosegregation of the X inactivation skewing trait with the ICF phenotype in an autosomal recessive mode. Inasmuch the ICF gene is probably involved in the methylation reaction, the nature of this transacting factor could be related to epigenetic modifications.

Program Nr: 1479 from the 1999 ASHG Annual Meeting

Genetic Characterization of a Diabetes Susceptibility Locus on Mouse Chromosome 4. *T.C. Brodnicki[1,2], P. McClive[1], S. Couper[1,2], G. Morahan[1,2]*. 1) Genetics and Bioinformatics , The Walter & Eliza Hall Institute, Melbourne, Victoria, Australia; 2) The Cooperative Research Center for Discovery of Genes for Common Human Diseases, Melbourne, Victoria, Australia.

Insulin-dependent diabetes mellitus (IDDM) is a multigenic autoimmune disease. The reason(s) autoreactive T cells become activated and mediate destruction of insulin-producing beta cells are not fully understood, but insights may be gained from one of the best animal models for IDDM - the nonobese diabetic (NOD) mouse. We previously located a NOD diabetes susceptibility locus, designated Idd11, on mouse chromosome 4 by analyzing diabetic backcross mice produced after crossing NOD/Lt with the nondiabetic resistant C57BL/6 (B6) strain. In order to confirm Idd11 and further refine its location, three NOD congenic mouse strains with different B6-derived intervals within chromosome 4 were generated. Two of the congenic strains had a significant decrease in the cumulative incidence of diabetes compared to NOD/Lt control mice. The third NOD congenic strain, containing a B6 interval surrounding the Slc9a1 locus, was not protected against diabetes. Mouse chromosome 4 has also been linked to another autoimmune disease, systemic lupus erythematosus (SLE), in the New Zealand Black (NZB) mouse strain. In order to further characterize Idd11, a mouse strain was generated which contained one copy of the NZB interval for Idd11 on the NOD background. Analysis of this mouse strain demostrated that the NZB interval for Idd11, compared to the B6 interval, is unable to protect NOD mice from diabetes. These combined results define a new distal boundary for Idd11, eliminate the Slc9a1 gene as a candidate and suggest that the Idd11 locus may play a role in SLE development.

Program Nr: 1480 from the 1999 ASHG Annual Meeting

Identification of a new polymorphism in intron 6 of the human *NURR1* gene and investigation of association with Parkinson's disease and schizophrenia. *S. Buervenich[1], A. Carmine[1], F. Xiang[2], Z. Zhang[2], O. Sydow[3], E.G. Jonsson[4], G.C. Sedvall[4], M. Anvret[2,5], L. Olson[1]*. 1) Dept. of Neuroscience, Karolinska Institutet, Stockholm, Sweden; 2) Dept. of Clinical Genetics, Karolinska Institutet, Stockholm, Sweden; 3) Division of Neurology, Karolinska Institutet, Danderyd, Sweden; 4) Dept. of Clinical Neuroscience, Psychiatry Section, Karolinska Institutet, Stockholm, Sweden; 5) AstraZeneca R&D Sodertalje, Sodertalje, Sweden.

Schizophrenia and Parkinson's disease have in common that affected relatives constitute the largest epidemiological risk factor, but the mode of inheritance of susceptibility is complex and remains to be elucidated. A further common observation in both diseases is etiologic and/or therapeutic involvement of dopaminergic neurotransmission. Recently, a number of studies have pointed out the importance of retinoids and retinoid-related genes for midbrain dopamine neurons. Mice lacking the retinoid-related orphan receptor *Nurr1* fail to develop mesencephalic dopamine neurons. We therefore considered the *NURR1* gene a good candidate for the two diseases. We sequenced the entire coding region and parts of all introns in 10 Parkinson patients, 10 schizophrenic patients and 4 controls. We identified a new polymorphism in the sixth intron that deletes a cleavage site for the restriction enzyme BseRI. This polymorphism was found to be present at a frequency of 20 to 25 percent in larger (n=140 alleles for each group) samples of controls, Parkinson patients and schizophrenic patients from Sweden. No significant differences were found between any of the groups. We conclude that strong association between the identified polymorphism and any of the two diseases can be excluded. However, larger materials have to be looked at in order to exclude minor effects of the identified sequence change.

Program Nr: 1481 from the 1999 ASHG Annual Meeting

Fully expanded fragile X CGG repeats exhibit a length- and differentiation- dependent instability in cell hybrids that is independent of DNA methylation. *R.W. Burman[1], B.W. Popovich[1], P.A. Yates[2], P.B. Jacky[3], J.L. Schnell[1], M.S. Turker[1,2]*. 1) Dept of Molecular and Medical Genetics, Oregon Health Sciences University, Portland, OR; 2) Center for Research on Occupational and Environmental Toxicology, Oregon Health Sciences University, Portland, OR; 3) Cytogenetics Laboratory, Kaiser Permanente NW, Clackamas, OR.

The fragile X syndrome is characterized at the molecular level by expansion and methylation of a potentially unstable CGG trinucleotide repeat located within the *FMR1* locus. Somatic instability occurs in the large majority of full mutation carriers resulting in repeat length mosaicism, which is manifest as a complex smeared and multi-banded hybridization pattern when examined by Southern blot analysis. These mutational patterns tend to be well conserved among multiple tissues within individuals and can be conserved in monozygous twins. Moreover, typical methylated full mutation alleles are stable in cultured fibroblasts. These observations suggest that repeat instability is limited to a period during early embryogenesis. The specific conditions that exist in embryonic cells which are believed permissive to fluctuations in repeat number have not been described.

To study the behavior of full mutation alleles in mitotic cells, we have generated human x mouse somatic cell hybrids that carry both methylated and unmethylated full mutation fragile X alleles. We analyzed repeat dynamics in these hybrids as a function of DNA methylation, repeat length, and cellular differentiation. We find that both methylated and unmethylated full mutation alleles are unstable in the hybrids. Repeat stability in the hybrids is dependent on repeat length except in an undifferentiated cellular background where large alleles are maintained with a high degree of stability. This stability is lost when the cells undergo differentiation. These results indicate that the determinants of repeat stability are more complex than generally believed and suggest an unexpected role for cellular differentiation in repeat stability.

Program Nr: 1482 from the 1999 ASHG Annual Meeting

Ethnic-Specific Allele Frequency Differences in the Human Interleukin-4 Receptor Gene. *M. Caggana, K. Walker, A.A. Reilly, J.M. Conroy, S. Duva, A.C. Walsh.* Wadsworth Ctr, New York State Dept Health, Albany, NY.

Two functionally significant sequence variants in the interleukin-4 receptor gene (*IL-4R*) were recently reported. One variant, a G to A transition at nucleotide 1902, results in the substitution of arginine for glutamine (Gln551Arg) and acts dominantly. The Arg variant is associated with atopy. The second recessively acting variant is an A to G transition at nucleotide 398. This results in the substitution of valine for isoleucine (Ile50Val). The Ile variant is associated with atopy and atopic asthma. Because asthma prevalence varies in different ethnic groups, we sought to examine the allele frequencies of these mutations in four anonymous New York State newborn screening populations unselected for disease (N=855). These data show that Arg551 is most frequently found in African-Americans with an allele frequency of 68%. However, the Ile50 allele is most common in Caucasians (allele frequency, 87%). When both alleles are studied together as a haplotype, a higher proportion of African-Americans were homozygous for the "enhanced signaling" variants (Ile50/Arg551). In concert with other signals, IL-4 binding to its receptor induces isotype switching in B cells from mu to epsilon and is therefore associated with increased IgE production. Therefore, our data suggest that the African-American population may be at increased risk for diseases, including asthma, which are associated with increased IgE production. In addition, these data emphasize the importance of determining the frequencies of single nucleotide polymorphisms (SNPs) in different populations before attempting to draw conclusions regarding allele association studies, since the background allele frequencies may be widely disparate between different populations. Studies to characterize two additional (*IL-4R*) sequence variants that result in amino acid substitutions in these same specimens and a cohort of phenotypically characterized unrelated asthmatics are underway.

Program Nr: 1483 from the 1999 ASHG Annual Meeting

Numerous Point Mutations in the Mitochondrially-Encoded Cytochrome c Oxidase Genes Detected by Single Strand Conformational Polymorphism Analysis of Brain Samples from Patients with Alzheimer's Disesase. *F.J. Castora, N.S. Hamblet, B. Ragland, M. Ali.* Dept Physiological Sciences, Eastern Virginia Med Sch, Norfolk, VA.

A “ mitochondrial hypothesis ” of late onset Alzheimer's Disease (AD) has been proposed based on studies that establish altered oxidative phosphorylation (OXPHOS) in AD tissue. Biochemical studies have indicated that there is a significant decrease in cytochrome c oxidase (COX) activity as well as perturbed COX I and COX III mRNA levels in platelets and in brain tissue from Alzheimer's patients. We have examined the mitochondrial-encoded COX subunits (COX I, II, and III) by single strand conformation polymorphism (SSCP) and DNA sequencing. The efficiency of SSCP in detecting mutations in the COX subunits was estimated to be 80 per cent. Twenty one mtDNA point mutations were identified. Seventeen of the mutations are new variants of the mitochondrial genome. One missense mutation in COX III at nucleotide position 9861 which was observed in four of thirty three autopsy-confirmed AD patients results in the alteration of a moderately conserved phenylalanine to a leucine. This 9861 mutation was absent in 26 control brains and is absent in a GENBANK search of 130 human mtDNA sequences examined. Measurements of cytochrome c oxidase activity in extracts from frozen brain samples indicated that there was a 31 per cent decrease in COX activity in AD brains compared to age-matched control brain samples, which agrees with previously published observations. COX activity in one of the AD brain samples carrying the 9861 mutation was decreased by 61 per cent relative to the activity obtained from control brain samples. These results suggest that this mutation may be linked to AD and that its phenotypic expression results in reduced respiratory complex IV activity.

Program Nr: 1484 from the 1999 ASHG Annual Meeting

Decreased frequency of a neutral sequence variant of the RET proto-oncogene in sporadic Hirschsprung disease.

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Hirschsprung (HSCR) disease is an inherited disorder characterized by absence of intrinsic ganglion cells in the distal gastrointestinal tract. Genetic mapping in humans and knock-out studies in mice have clearly shown that different susceptibility genes, involved in either the RET or the endothelin signalling pathways, contribute to HSCR phenotype. Interestingly, alterations of these genes are detected in only 30-50% of all HSCR patients, suggesting the involvement of modifier genes and/or additional genetic or environmental risk factors. We have analyzed about 300 HSCR patients confirming that a wide range of mutations of the RET proto-oncogene accounts for the highest proportion of cases. In addition to causative nucleotide changes, we have identified rare sequence variants and neutral substitutions which might act as susceptibility alleles conferring an altered disease risk. To test this hypothesis, we have retrospectively compared the frequencies of RET alleles detected in 52 Italian sporadic HSCR patients with respect to those found in 92 population-matched unaffected individuals, finding that a C2508T transition in exon 14 of the RET gene (S836S) is clearly less frequent in HSCR patients than in controls and the difference is statistically significant (Fisher exact test p=0.004). In agreement with a recent report (Gimm et al.,1999), we found the same variant is over-represented in patients with sporadic medullary thyroid carcinomas (MTC). These observations suggest that codon S836 may play an important role on normal RET function, inducing opposite functional effects when/if its sequence is altered. So far, we have excluded a role of the C2508T variant in RNA processing and DNA-protein interaction, while a possible linkage disequilibrium with another RET alteration or an effect on RNA translation are currently under investigation.

Program Nr: 1485 from the 1999 ASHG Annual Meeting

Genes on human chromosome 18q21 identified using exon trapping: towards the determination of bipolar susceptibility loci. *H. Chen[1], D. DePaulo, Jr.[1], G.N. Rochino[1], Y. Huo[1], T. Swift-Scanlan[1], C.A. Ross[1,2], M.G. McInnis[1], P. Sklar[3]*. 1) Department of Psychiatry & Behavioral Sciences; 2) Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21287, U.S.A; 3) Massachusetts General Hospital, Boston, MA.

The region between D18S41 and D18S60 in the q21 band on chromosome 18 (HC18) has been linked to bipolar disorder (Stine et al, 1995; McMahon et al, 1997). In order to identify genes systematically in this region we performed exon trapping on cosmids which had been isolated from an HC18-specific cosmid library (LL18NC02) using 47 STSs from the interval as hybridization probes. A total of 285 unique sequences (exons) were obtained. Homology searching of the databases using NCBI's BLAST algorithms revealed that 32 exons show identity to known genes and/or ESTs, 29 have significant homologies to genes from human and other species, and 19 sequences are homologous to ALU or other repeats. There are 205 sequences (72%) with no homology to entries in public databases. Of these exons, 34 were selected at random for mapping back to chromosome 18. Twenty-nine of 34 exons tested were mapped back to chromosome 18 using PCR amplification of the Genebridge4 radiation hybrid panel. Sixteen exons localized to the 18q21 region. We performed cDNA library screening and RT-PCR analysis to test whether the exons are expressed. Three pools of 6 exons each identified over 120 cDNA clones from a human fetal brain library. RT-PCR products from fetal brain RNA were obtained from 26 of the 34 exons (76%). The majority of the exons are being mapped to chromosome 18. We are developing full length cDNAs according to the trapped exons localized to the region and single nucleotide polymorphisms for association studies in bipolar families. A comprehensive transcript map in the region will provide valuable tool for the identification of susceptibility genes for bipolar illness and other disorders that map to the chromosome.

Program Nr: 1486 from the 1999 ASHG Annual Meeting

Association of gene markers on chromosome 5q and 11q with asthma in a Chinese population. *H. Chen[1,2], Y.Zh. Chen[2], L.P. Hu[3], J. Fu[2], H.Q. Zhang[2].* 1) Molecular Biochemistry, Basic Medical Science, Beijing, P.R.China; 2) Capital Insitute of Pediatrics, Beijing, China; 3) Academic Medical Science of Armyä.

We investigated linkages of asthma phenotypes to the markers on chromosome 5q31-33 and 11q13 in order to accumulate data on the related loci in the Chinese population. Thirty-two families with 192 samples were collected from 3 villages in the Fujian province of Southern China which has the highest morbidity (5%) of asthma reported from a recent epidemiological study. Each family was selected through the proband, a child with symptomatic asthma. The polymorphic markers D5S436 and D5S393 at chromosome 5q31-33 were genotyped. Sib-pair analysis showed evidence for linkage of asthma with D5S436 (IBD t=2.714, P<0.05, IBS t=2.313, P<0.05) but not D5S393. No significant linkage to total serum IgE was observed with either marker. Multiallelic TDTs was performed using the program ETDT. Transmission for individual alleles revealed the strongest association of asthma with particular allele of D5S436 (allele 236bp, ratio of transmitted: not transmitted 42:20, P=0.0052). Allele 236 of D5S436 was also found to be transmitted more frequently to offspring with high total serum IgE (20:7, P=0.0124). A coding variant of E237G and two RsaI polymorphic sites in the non-coding region of the FceRI-b gene (chromosome 11q13) were conducted. The allele frequencies for three FceRI-b gene polymorphisms in the Chinese population were markedly different from the published results of Caucasians and Japanese. The MIXED procedure by SAS system was used to evaluate differences in several factors-specific means for total serum IgE. The only significant difference was found between genotype of RsaI polymorphic site in intron 2 and total serum IgE levels. Further analysis of association between A allele of intron 2 polymorphism and elevated total serum IgE was also significant (Fish exact P=0.039, RR=1.316, 95%CI=1.028-1.803, P=0.038). However, biallelic TDT showed strong evidence for linkage disequilibrium between allele G of E237G and asthma (c^2td=10.24, P<0.01). These findings suggest that chromosome 5q31-33 and 11q13 may still be very attractive candidates for further investigation in the Chinese population.

Program Nr: 1487 from the 1999 ASHG Annual Meeting

Enzymatic and NMR spectral evidence of hairpin stability in DNA triple repeats: a possible mechanism for initiating the repeat expansion. *S.-H. Chen[1], C.R. Scott[1], S.-H. Chou[2]*. 1) Department of Pediatrics, University of Washington, Seattle, WA; 2) Institute of Biochemistry, National Chung Hsing University, Taichung, Taiwan, ROC.

Hairpin formation of single strand trinucleotide DNA repeats during meiosis has been suggested to be an event that possibly leads to the repeat expansion being associated with specific neurological diseases. We have synthesized pure CGG, CCG, CAG, CTG tandem repeats, and repeats with specific interruptions. The following experiments were performed: 1) Analysis of homoduplex and hairpin formation of the oligonucleotide repeats, 2) Hairpin stability test by an enzymatic assay (T4 DNA polymerase) of the repeats, and 3) Secondary structure stability analysis of the repeats by NMR imino proton spectra. We found that 1) Both hairpin and self-complimentary structures were detected for the CGG repeat under different conditions,while only hairpin was observed for the CGG, CAG and CTG up to 9 pure repeats. 2) T4 DNA polymerase studies showed that hairpin structures of $(CGG)_7$ and $(CCG)_7$ repeats were stable, while the repeats with interruptions were not. 3). NMR spectra revealed that the temperatures for imino proton "melting" of the $(CCG)_7$ and $(CGG)_7$ tandem repeats were greater than 50° C, while those for the $(CGG)_5\underline{T}CGCCG$ and $(CGG)_3\underline{A}GG(CGG)_3$ interrupted repeats were less than 40° C. These results suggest that a stable hairpin structure might be the initiating step enabling the expansion of the repeats.

Program Nr: 1488 from the 1999 ASHG Annual Meeting

A novel 2 bp deletion in Cytochrome C oxidase subunit II (COX II). *T. Chen[1], W. Fan[1], M. Lipson[2], M. Sifry-Platt[2], A. Grix[2], L. Wong[1]*. 1) Inst. for Mol & Human Genetics, Georgetown University, Washington, DC; 2) Kaiser Permanente Genetics Dept. 1650 Response Road Sacramento, CA 95815.

Most cytochrome oxidase (COX) deficiencies are autosomal recessive disorders associated with a variety of syndromes including Leigh and fetal infantile myopathy. COX deficiency has also been found in mitochondrial DNA (mtDNA) disorders with point mutations in tRNAs or large mtDNA deletions. Mutations in mitochondrial mRNA encoding COX subunits have only been reported once in COXIII (an inframe deletion of 15 bp) and once in COX II (mutation at initiation codon). We report here, for the first time, a 2 bp deletion in COX II, resulting in frame shift, truncated COX II mutant subunit. The mutation was found in a newborn male died at 12 days of age following a course of apnea and bradycardia, and 2 days of metabolic deterioration with significant lactic acidosis (serum lactate: 54 mmol/L; normal: 0.5-2.2 mmol/L). A twin brother also decompensated around the same time and expired. Family history was remarkable for a maternal half sibling died at 2 months of age following a DPT vaccination. The heteroplasmic mutation was first detected in a skeletal muscle autopsy by Temporal Temperature Gradient Gel Electrophoresis (TTGE). DNA sequencing analysis revealed a 2 bp deletion at 8042-8043 del AT (or 8043-8044 del TA). This mutation results in an alteration of three amino acids followed by a premature stop codon. The mutant COX II is 68 amino acids shorter than the wide type protein. Subsequent PCR/ASO analysis demonstrated that same heteroplasmic mutation was present in the autopsy tissues of heart muscle and liver, as well as in the tissue cultures of skin and lung, but absent in the mother blood sample.

Program Nr: 1489 from the 1999 ASHG Annual Meeting

Mutation analysis of spinocerebellar ataxia type 1 gene in Taiwanese schizophrenic patients. *Y.H. Chen[1], C.H. Chen[1,2]*. 1) Institute of Human Genetics, Tzu-Chi Medical College, Hualien, Taiwan; 2) Department of Psychiatry, Tzu-Chi General Hospital, Hualien City, Taiwan.

Schizophrenic is a devastating, chronic mental disorder with genetic component in its etiology. Previous linkage study suggested chromosome 6p22-24 may harbor important gene(s) for schizophrenia. Spinocerebellar ataxia type 1 (SCA1) gene was mapped to this region and was considered as a putative candidate gene for schizophrenia. In addition, the SCA1 gene contains polymorphic CAG repeats in its coding sequences, and evidence of linkage disequilibrium between schizophrenia and SCA1 CAG repeat was also reported by researchers, suggesting the possible important role of SCA1 gene in schizophrenia. Recently, Pujana and his colleagues reported identification of a serine to cysteine at codon 186 (S186C) in a schizophrenic from Spain (Hum Genet 1997;99:772-775), which added further support that SCA1 gene may play a role in the pathology of schizophrenia. To test if the SCA1 gene also involves in the pathogenesis of schizophrenia in our population, we first determined the CAG repeats number in a cohort of 115 schizophrenic patients and 87 non-psychiatric controls. The CAG repeats range from 21 to 34 in both groups, neither abnormal expansion of CAG repeats was observed in our patient, nor significant different CAG genotypes distributions was observed between patients and controls. We further systematically searched for the mutations in the protein coding sequences of SCA1 gene in 63 Chinese schizophrenic patients with positive family history. Currently we identified two polymorphisms of SCA1 gene in our population, including 1865T/C and 2150A/G, which were already reported in literature. However, we did not identify any other mutation of SCA1 gene in this cohort, including the S186C mutation. Hence, we suggest that SCA1 gene may not play a major role in the pathogenesis of schizophrenia in our population.

Program Nr: 1490 from the 1999 ASHG Annual Meeting

Determination of microsatellite total allele content differences between DNA pools: applications to association and population studies. *H.E Collins[1], S.E. Inda[1], C.M. Phillips[1], J. Tuomilehto[2], M.F. Seldin[1]*. 1) Rowe Program,UC Davis, Davis, CA 95616; 2) Nat. Public Health Inst. Helsinki, Finland.

DNA pooling is a potential tool for genome wide association studies or population comparisons. A simple method for measuring total allele differences in comparisons between two pools containing large numbers of DNA samples is presented. The method compares relative peak height differences between electrophoretigrams for each allele of a microsatellite. The method was evaluated by analysis of eleven microsatellites utilizing pooled sample sizes of 50, 100 and 200 individual DNA samples. Calculated pool differences were compared with real total allele differences determined by individual genotyping. Together > 200 comparisons demonstrated a correlation coefficient of 0.96. This method can provide a rapid screen for total allele differences of greater than 5-10%, a threshold that should be applicable to detecting low relative risk genes in common diseases. The validity of this method for association screens was verified by comparing a pool of 200 Finnish probands with rheumatoid arthritis to 200 Finnish controls. A strong positive signal was obtained for three MHC linked microsatellites, and allele specific differences were consistent with those anticipated for the DRB1 shared epitope. The applicability of the method to population comparisons was demonstrated by comparing microsatellite allele frequencies for an array of microsatellites between 5 ethnic pools, each with ³300 individuals. For analysis of >50 markers, the mean intrapool difference was 5.7%. In contrast, differences >20% were found for 50% of the markers between European Americans (EA) and Africans and 30% of the markers between EA and African Americans (AA), but only 8% of markers between EA and Hispanic Americans (HA). These findings demonstrate larger EA admixture in the HA than AA ethnicity. In conclusion, these studies suggest that DNA pooling can be a powerful tool in association studies for the determination of candidate regions for a range of complex genetic diseases, and in population screens to determine evolutionary divergence or to screen for markers useful for mapping by admixture disequilibrium.

Program Nr: 1491 from the 1999 ASHG Annual Meeting

Parent-of-Origin Effects in the Disequilibrium Mapping of Quantitative Traits. *W.O.C. Cookson, G.R. Abecasis, L.R. Cardon.* Wellcome Trust Center for Human Genetics, University of Oxford, OX3 7BN, United Kingdom.

Gene expression is the end result of complex and diverse regulatory mechanisms. It has been observed that for some genes expression of maternally and paternally inherited copies is differentially regulated, and it is likely that some of the genes underlying complex disease may be subject to parent-of-origin effects, such as methylation mediated imprinting. When the effect of maternal and paternal alleles is not modeled separately, traditional methods of association and linkage mapping can be inefficient in locating imprinted genes. For family-based association analysis of quantitative traits, we describe how imprinting effects can be modeled and distinguished from maternal effects and population stratification, and how the difference between the effects of maternal and paternal alleles on a particular trait can be evaluated.

For a range of effect sizes and allele frequencies, we examine the power to detect significant differences between models that allow for parent-of-origin effects and those that do not. We show that when the effect of maternal and paternal alleles differs greatly, models that do not allow for parent-of-origin effects may be unable to locate imprinted genes at all. Modeling the effect of maternally and paternally inherited alleles separately can significantly increase power. Allele frequencies appear to be especially important for di-allelic markers, as the parental origin of the inherited alleles may often be ambiguous. We show that, in sets of di-allelic markers, power can be greatly increased when haplotypes are used to resolve cases of ambiguity between maternal and paternal inheritance. These results and methodology may be of general value in the dissection of complex human disease.

Program Nr: 1492 from the 1999 ASHG Annual Meeting

Glaucoma phenotype associated with the GLC1A Gln368STOP mutation from the Glaucoma Inheritance Study in Tasmania. . *J.E Craig[1, 3], D.A. Mackey[1,3], P. Baird[1], A.I. McNaught[1,3], J.L. Dickinson[3], J.L. Rait[1], M.A. Coote[1], D.L. Healey[1], J.H. Fingert[2], E.M. Stone[2]*. 1) CERA, Melbourne University, East Melbourne, Victoria, Australia; 2) Department of Ophthalmology,University of Iowa,Iowa City; 3) Menzies Centre, University of Tasmania, Hobart, Australia.

Purpose: To investigate the age-related penetrance and phenotypic variability of Primary Open Angle Glaucoma (POAG) in Australian families with the GLC1A mutation Gln368STOP. Methods: Pedigrees with the Gln368STOP mutation in the myocilin gene (MYOC) at the GLC1A locus were identified from 1900 cases of POAG in Tasmania. Individuals positive for the Gln368STOP mutation were identified and examined along with available family members. Results: Gln368STOP is the commonest glaucoma mutation identified in our population to date. Incomplete penetrance was observed. Family history of POAG on both maternal and paternal sides, and phenocopies were frequently observed. Conclusions: The Gln368STOP mutation is associated with POAG of variable age of onset. Other factors, as yet uncharacterised, may be involved in expression of the POAG phenotype in Gln368STOP pedigrees.

Program Nr: 1493 from the 1999 ASHG Annual Meeting

Novel repeat structures identified in African-Americans represent a new mechanism leading to fragile X CGG repeat instability. *D.C. Crawford[1], C.E. Schwartz[2], S.T. Warren[1,3], S.L. Sherman[1].* 1) Department of Genetics, Emory University, Atlanta, GA; 2) P.C. Self Research Institute, Greenwood Genetic Center, Greenwood, SC; 3) Howard Hughes Medical Institute, Atlanta, GA.

The fragile X syndrome is caused by an unstable CGG repeat in the 5' UTR of FMR-1. To date, a number of factors have been identified which may influence repeat instability including number and position of interspersed AGGs, length of the 3' pure CGG repeat, and haplotype. However, nearly all such data were derived from studies of Caucasian populations (CC). Here we examine the African-American population (AA) by haplotyping a large, unaffected (n=643) and fragile X (n=63) AA population and sequenced the CGG repeat array in a subset of these chromosomes (n=125). Compared with the established CC data, the unaffected AA population had a significantly decreased frequency of intermediate alleles (IA; 41-60 repeats) yet the same prevalence of the syndrome. Furthermore, there was no association of haplotype background with these IAs in contrast to that observed among CCs. Sequencing data show that the frequency of alleles with >24 pure repeats at the 3'end of the repeat in the AA population (0.03) was similar to that shown in CCs. Therefore, although there was a decreased frequency of IAs in the AA population, the frequency of susceptible repeat structures was similar to the CC population. Among the fragile X AA, we identified a unique haplotype background not yet observed in the unaffected CC population. Also, this haplotype background was rare in the unaffected AA population and was not found among the IAs. Sequencing of these alleles revealed the lack of a 5' AGG but the retention of a 3' AGG. These data suggest that the lack of a 5' CGG repeat may be a factor for repeat instability. We propose that these repeat structures increase the risk for either 1) loss of the 3' AGG leading to an allele of >24 pure repeats or 2) expansion of the number of 5' CGG repeats. Either result may lead to rapid expansion to the premutation. To distinguish between the two possible mechanisms, premutation alleles of this haplotype background are being sequenced to determine the presence or absence of a 3' AGG.

Program Nr: 1494 from the 1999 ASHG Annual Meeting

A low enzyme activity haplotype at DBH associates with cocaine-induced paranoia. *J.F. Cubells[1,2], H.R. Kranzler[3], E. McCance-Katz[1], G.M. Anderson[4], R.T. Malison[1], L.H. Price[1], J. Gelernter[1,2].* 1) Dept Psychiatry, Yale Univ Sch Med, New Haven, CT; 2) VACHS, West Haven, CT; 3) Dept. Psychiatry, Univ. Conn. Health Center, Farmington, CT; 4) Yale Child Study Center, New Haven, CT.

Low levels of dopamine b-hydroxylase (DbH) protein in the serum or cerebrospinal fluid (CSF) are associated with greater vulnerability to positive psychotic symptoms across several diagnostic groups. DbH level is a stable, genetically controlled trait. The locus encoding DbH protein, DBH, is the major quantitative trait locus controlling plasma and CSF DbH levels. We therefore hypothesized that DBH variants associated with low levels of DbH in the plasma would also associate with greater vulnerability to cocaine-induced paranoia (CIP).

To test this hypothesis, we first showed that a di-allelic variant, DBH5'-ins/del, located approximately 3 kb 5' to the DBH transcriptional start site, significantly associates with plasma DbH activity in European-Americans (EAs; N = 66). Linkage disequilibrium (LD) analysis of that polymorphism and DBH444g/a, another di-allelic variant associated with DbH activity, demonstrated that alleles of similar association to DbH levels are in positive LD. We then estimated DBH haplotype frequencies in cocaine-dependent EAs rated for cocaine-induced paranoia (N = 45). As predicted, the low-DbH-associated haplotype, Del-a, was significantly more frequent ($p = 0.0005$) in subjects endorsing CIP (N = 29) than in those denying CIP (N = 16). Comparison to haplotype frequencies in control EAs (N = 170) showed that the association predominantly reflected under-representation of Del-a haplotypes in those denying CIP.

We conclude that (a) the two DBH polymorphisms we studied are associated with DbH activity; (b) those two polymorphic systems are in significant LD in EAs, with alleles of similar association to DbH levels in positive LD, and (c) the haplotype associated with low DbH activity is associated with CIP.

Program Nr: 1495 from the 1999 ASHG Annual Meeting

Identification of six mutations in the *Caspase-8* gene in non-obese diabetic (NOD) mice. *A. Davoodi-Semiromi, M. Marron, E. Leung, C-Y. Wang, J-X. She.* Department of Pathology, University of Florida, Gainesville, FL. USA, 32610.

Apoptosis is a major form of cell death characterized initially by a series of morphological changes. This process appears to play an important role in the development of diabetes in the non-obese diabetic mice (NOD). Key elements that initiate and eventually execute the apoptotic program are members of the caspase family of cystein protease whose activation is believed to be essential for virtually all forms of apoptosis. Here we report the identification and characterization of six mutations in the caspase-8 gene in mice. Total RNA was extracted and converted to the cDNA by reverse-transcription and amplified by polymerase chain reaction (RT-PCR). The whole coding sequence and parts of the 3' and 5' end of the gene were amplified and subjected to direct sequencing. We sequenced the gene for NOD and six other strains and we found the same mutations in NOD, MRL and Balb/c. These mutations, however, were not present in B6, C3H, CBA and NZW. An A to G at position -23 and an Ala96Val along with four silent mutations at codons 48, 92, 188, and 317 were found. Of six mutations identified in this study, the A to G transition at position -23 and a missense mutation at codon 96 (Ala96Val) might be pathological mutations. Our data show that these mutations, however, are not specific for NOD mice since they are found in other strains as well. Functional significance of these mutations and their potential role in IDDM remains to be elucidated.

Program Nr: 1496 from the 1999 ASHG Annual Meeting

Molecular characterization of four endogenous retroviral related sequences identified by Representational Difference Analysis from three sets of monozygotic twins discordant for schizophrenia. *P. Deb-Rinker[1], P.P. McDonald[1], M.J. Lewis[1], R.L. O'Reilly[2], S.M. Singh[1]*. 1) Molecular Genetics Unit (Zoology), University of Western Ontario, London, Ontario, Canada; 2) Psychiatry, University of Western Ontario, London, Ontario, Canada.

A significant proportion of the human genome consists of sequences with a retroviral origin, some with the capacity to transpose. Such human endogenous retroviral sequences could cause mutations by insertions as well as provide a basis for the evolution of the human genome. Despite the notable representation of retrovirus-like sequences in the human genome, relatively little is known about their organization, distribution and significance. We have isolated four retroviral-related sequences from human genomic DNA representing three sets of monozygotic twins discordant for schizophrenia by Representational Difference Analysis. Southern blot analysis has identified one of them (MSRV-like gb AF009668) to be present in multiple copies (15-20) while the other three (with no significant database match) are single copy sequences. The mutlicopy MSRV-like sequence was FISH mapped to at least six different chromosomal locations, suggesting that HERVs have a dispersed pattern in the human genome with few chromosomal sites favoring integrations. The origin of these sequences was assessed with a Zooblot. All four sequences were limited to humans and rhesus monkey lanes suggesting a primate lineage. It is interesting that three of these sequences showed weak positive signals in the Bovine lane, indicating a related retroviral element in this species. Further, when used as probes on Northern blots, two of these sequences identify 7-9kb RNA transcripts in the human placenta. The other two sequences also identify RNA transcripts in a few adult tissues. These results on retroviral elements bring out the potential of these and related sequences in the human genome as causative agents for insertional mutagenesis during development and differentiation. Such mechanisms could cause significant pathological effects and contribute to discordance of monozygotic twins for complex multifactorial diseases including schizophrenia.

Program Nr: 1497 from the 1999 ASHG Annual Meeting

A common substitution (C-514T) in the human hepatic lipase gene promoter decreases its transcriptional activity. *S.S. Deeb.* Dept Genetics/Medicine, Univ Washington, Seattle, WA.

Hepatic lipase catalyzes the hydrolysis of triglycerides and phospholipids in plasma high-density lipoprotein (HDL) and low-density lipoprotein (LDL) particles. Four polymorphisms exist in the proximal promoter of the human hepatic lipase gene (LIPC). They are in almost complete linkage disequilibrium. The less common haplotype, which occurs at a frequency of between 0.15-0.21 among Caucasians, is associated with decreased postheparin plasma hepatic lipase activity, increased plasma HDL cholesterol (particularly the phopholipid-rich HDL2 particles) and increased bouyancy of circulating LDL particles. Decreased HDL cholesterol and prevalence of small dense LDL particles constitute a risk factor for atherosclerosis. We investigated whether any of the four LIPC promoter polymorphisms influences transcriptional activity of proximal promoter constructs (-639/+29) as measured by transient transfection of the murine heptocyte cell line ML12. We observed that the C-514T substitution resulted in approximately a 30 per cent decrease in promoter activity ($P<0.0001$). On the other hand, the G-250A substitution had no effect on activity of promoters with either T or C at position -514. Interestingly, the substitution at position -514 lies within a potential binding site for a transcription factor (upstream-stimulatory factor)that has been shown to mediate insulin action on gene transcription. In conclusion, we postulate that the C-514T substitution contributes significantly to the decrease in levels of plasma hepatic lipase activity, which in turn results in higher levels of phospholipid-rich HDL2 and large bouyant LDL particles.

Program Nr: 1498 from the 1999 ASHG Annual Meeting

Association between schizophrenia and the UFD1L promoter polymorphism - 277A/G. *A. De Luca[1], A. Botta[1], F. Amati[1], E. Conti[1], A. Pasini[2], F. Macciardi[3], N. King[3], G. Spalletta[2], B. Dallapiccola[1,4], G. Novelli[1]*. 1) Department of Biopathology and Diagn. Imaging, Tor Vergata University and CSS Mendel Institute, Rome,Italy; 2) Department of Psychiatry, Tor Vergata University, Rome,Italy; 3) Neurogenetic Section, Clarke Institute of Psychiatry, University of Toronto, Ontario, Canada; 4) IRCCS,CSS,S.Giovanni Rotondo, Italy.

Haploinsufficiency resulting from deletion of a gene or a number of genes at 22q11.2 has been associated with a variety of clinical disorders, including DiGeorge syndrome (DGS) and velocardiofacial syndrome (VCFS). Hemizygosity of 22q11.2 has been also associated with schizophrenia or schizoaffective disorders. To identify the schizophrenia susceptibility gene(s) in this region, we evaluated a novel single nucleotide polymorphism mapped within a region with functional promoter activity of the Ubiquitin Fusion Degradation Protein-1 (UFD1L) gene. UFD1L gene is considered a candidate for schizophrenia, since it is expressed during development in the medial telencephalon that forms hippocampus and is deleted in DGS/VCFS patients most of which develop psychiatric disorder by adult age. Sixty-one individuals with DSM-IV--SCID-P schizophrenia and 70 ethinically matched controls were genotyped for the -277A/G UFD1L promoter polymorphism. A significant increase in the frequency of the -277A variant allele of the UFD1L promoter polymorphism was found in schizophrenic patients (0.54) compared to matched controls (0.35) ($P<0.003$). The difference in genotype distribution was also significant ($P<0.02$).To elucidate the role of this gene in the pathogenesis of schizophrenia expression and protein localization in the human brain were investigated. Northern and western blot analyses using mRNA and protein extracted from different areas of adult human brain, confirmed the presence of the UFD1L transcript and protein in the brain expecially in the cerebellum, cerebral cortex, and thalamus. These results support the presence of a susceptibility locus for schizophrenia in chromosome 22. Work supported by a grant from Italian Ministry of Health and Telethon (N. E 723 and 264/bi).

Program Nr: 1499 from the 1999 ASHG Annual Meeting

Maternal Multivitamin Use, Genetic Variation in Folate Metabolism and Risk of Oral Clefts. *S.R. Diehl, M.H. Khoshnevisan, D.F. Wyszynski, T. Wu, A. Miller-Chisholm.* Craniofacial Epidemiology and Genetics Branch, NIDCR/NIH, Bethesda, MD, USA.

The purpose of this study was to evaluate the association between SNPs at the MTHFR gene, maternal multivitamin consumption and risk of nonsyndromic cleft lip with or without cleft palate in a Caucasian population. We studied 234 families with one or more CL/P affected members. Two SNPs in the MTHFR gene (C677T and A1298C) were assayed by standard PCR-RFLP techniques. We performed a transmission disequilibrium test and a case-only analysis to evaluate linkage disequilibrium (association) and interactions with maternal multivitamin consumption. Without considering multivitamin consumption, we found significant (1) < 0.05) evidence of disequilibrium with risk of cleft lip for the C677T SNP, but no association with the A1298C polymorphism for all subjects. However, when we incorporated multivitamin consumption into our analyses, we found a much stronger association of CL/P risk for the C677T MTHFR SNP for mothers who did use multivitamins during their first trimester of pregnancy ($p < 0.005$). We also found a weak association with the A1298T MTHFR SNP for mothers who did not use multivitamins. These findings indicate the presence of a strong gene by environment interaction for MTHFR SNPs and maternal multivitamin consumption.

Program Nr: 1500 from the 1999 ASHG Annual Meeting

FMRP is a phosphoprotein and a substrate of the Fes non-receptor tryosine kinase. *S.P. DiMarco, S. Ceman, E. Torre, S.T. Warren.* Howard Hughes Medical Institute and Departments of Biochemistry, Pediatrics and Genetics, Emory University School of Medicine, Atlanta, GA 30322.

Fragile X syndrome, a common form of mental retardation is caused by the absence of FMRP, the encoded protein of FMR1. FMRP is an RNA binding protein and shuttles between the cytoplasm and nucleus. Phosphorylation of certain proteins has been shown to modulate RNA binding and cellular localization, therefore, we decided to investigate the phosphorylation status of FMRP. Here we describe FMRP as a serine, threonine and tyrosine phosphoprotein. We have pursued the tyrosine phosphorylation of FMRP and identified the Fes kinase as responsible for the tyrosine phosphorylation of FMRP in vivo and in vitro. The tyrosines phosphorylated by Fes were identified by CNBr cleavage and 2-dimensional peptide mapping of Fes phosphorylated FMRP. Three tyrosine residues within an 8kDa CNBr fragment of FMRP, adjacent to the nuclear export signal, were found to be phosphorylated. These tyrosines were mutated to phenylalanines and the protein was no longer able to be phosphorylated by Fes in vivo. Similar to other substrates of Fes, FMRP was found associated with Fes in immunoprecipitations. NIH 3T3 cells, transiently expressing Fes, were labeled with 32P-orthophosphate, nuclear and cytoplasmic lysates were made and immunoprecipitated with an anti-FMRP antibody. Using phosphoamino acid analysis, FMRP was found to be tyrosine phosphorylated only in the nucleus. Nuclear FMRP only accounts for 4-5% of cellular FMRP, which correlates with the amount of FMRP found to be tyrosine phosphorylated in normal lymphoblastoid cells. The three tyrosines phosphorylated by Fes may play a role in RNA binding, mRNP assembly, or nuclear transport of FMRP.

Program Nr: 1501 from the 1999 ASHG Annual Meeting

The subcellular distribution of HTm4, a hematopoietic specific protein and a canditade gene for atopy. Identification of the CDK-associated phosphatase KAP as an interacting protein. *J.L. Donato, H.B. Cai, C.N. Adra.* Beth Israel Deaconess Med Ctr, Harvard Medical School, Boston, MA.

HTm4 is a four transmembrane protein expressed specifically in hematopoietic cell lineages. This protein is 214 amino acids in length, with a predicted molecular weight of 25 kDa and is significantly (28%) homologous to CD20 and FceRIb. The HTm4 gene is organized into 7 exons and 6 introns, whose structure is reminiscent of the FceRIb and is localized to chromosome region 11q13.1, closely clustered with CD20 and FceRIb. We have identified two independent loci for atopic asthma on 11q13.1. One is for atopy (high IgE levels) and lies in a region that includes FceRIb and HTm4 genes, the other is for asthma per se (wheeze and labile airway obstruction) and lies close to D11S480. HTm4 is expressed in G0 hematopoietic stem cells, and in cells committed to differentiate into myeloid and B lymphocyte cell lineages. The gene is "shut off" in non-quiescent stem cells that are undergoing proliferation and differentiation. We used a specific polyclonal antibody to study the cell localization and protein interactions first detected by the yeast-two hybrid assay. Flow cytometry and immunohistochemistry analysis using the anti-HTm4 antibody showed an intracellular localization of HTm4 in U937 cells. A prominent immunofluorescent staining was seen around the nuclei. After U937 nuclei purification and Western blotting analysis, strong HTm4 signals were obtained in both total cell lysate and membrane fractions, but no significant reaction was observed in the nuclei enriched fraction, demonstrating that HTm4 is mainly located in the ER membranes. The yeast assay revealed that HTm4 specifically binds KAP (CDK-Associated Phosphatase). This interaction was confirmed by Western blotting analysis showing that HTm4 co-immunoprecipitates with KAP in total cell lysates incubated with anti-KAP antibody. Since KAP modulates the activity of the cyclin dependent kinases cdk2 and cdc2, our results emphasize the importance of HTm4 gene in hematopoietic cells and open the possibility that this molecule participates in the cell cycle events and in the maintenance of cells of the immune system.

Program Nr: 1502 from the 1999 ASHG Annual Meeting

Analysis of C. elegans transgenic for HD gene constructs with an expanded repeat. *J.C. Dorsman[1], H.G.A.M. van Luenen[3], M.A. Smoor[1], B. Pepers[1], M. Bremmer-Bout[1], J.T. den Dunnen[1], R.H.A. Plasterk[3], R.A.C. Roos[2], G.J.B. van Ommen[1]*. 1) Department of Human Genetics, LUMC, Leiden, The Netherlands; 2) Department of Neurology, LUMC, Leiden, The Netherlands; 3) The Netherlands Cancer Institute, Division of Molecular Biology, Amsterdam, The Netherlands.

Huntington's disease (HD) is a neurodegenerative disorder with a midlife onset. The disease is caused by expansion of a CAG (gln) repeat within the coding region of the HD gene. We are studying the effects of expanded huntingtin repeats in the nematode C. elegans, an inexpensive model animal, of which the neuroanatomy, differentiation and genetics are well-defined. We have generated nematodes transgenic for exon1 or exon1-7 of the HD gene, with (CAG)17, (CAG)73 or (CAG)140 repeats. The transgenes are present either as an extra-chromosomal array or integrated into the genome. Expression of the transgenes is driven by the HSP promotor which is regulated by heatshock. After heatshock induction a clear expression of the transgenes can be detected. Expression of the 140 HD transgene could only be detected with a monoclonal antibody specifically recognizing expanded gln repeats and with polyclonal antibody CAG53B which recognizes exon1 sequences. An extensive survey of transgenic worms containing the transgenes on extrachromasomal arrays revealed that a single heat shock of either eggs or L1 larvae has no effect on the percentage of transgenic offspring. Preliminary experiments suggest that expression of long gln repeats decreases the lifespan considerably. Transgenic animal and cell-model studies for HD have been hampered by the intrinsic instability of long CAG-repeat sequences in sub-cloning experiments. We have now shown that this stability problem can be reduced by the interruption of the perfect repeat at the DNA level by an alternative trinucleotide sequence encoding the same amino acid, i.e. a mixed repeat consisting of CAG and CAA triplets. We will use these larger repeat sequences (e.g. 300 gln) to accelerate the disease process and/or to cause more severe (lethal) phenotypes facilitating, e.g., high throughput drug-testing studies.

Program Nr: 1503 from the 1999 ASHG Annual Meeting

Genome-wide linkage analysis in a New Zealand family with familial Vesicoureteric Reflux. *M.R. Eccles[1], G.J. Jacobs[1], K.L. Choi[1], M. French[1], L. McNoe[1], J. Goodwin[1], S. Edwards[2], P. Tomlinson[2], G.D. Abbott[3]*. 1) Dept Biochemistry, Univ Otago, Dunedin Otago, New Zealand; 2) Dept Paediatrics, Univ Otago, Dunedin Otago, New Zealand; 3) Dept Paediatrics, Christchurch School of Medicine, Univ Otago Christchurch, New Zealand.

Vesicoureteric reflux (VUR) is very common in children, with an estimated incidence of between 0.4% and 9% of the general population. Approximately 15% of end-stage renal failure in children is caused by VUR making it one of the commonest causes of end-stage renal failure. The strong familial association of VUR and associated reflux nephropathy suggests that VUR may have a genetic component. Previous studies have suggested (one) dominantly inherited locus of major effect. PAX2 mutations cause VUR in a quarter of individuals with renal-coloboma syndrome, however PAX2 mutations are absent in patients with primary familial VUR. We have now carried out genome-wide linkage analysis in a 4-generation New Zealand family with familial VUR, in which 7 of 35 individuals were affected. Parametric and non-parametric programmes were used to analyse 226 markers evenly spread over the genome. Six regions were obtained on chromosomes 2, 4, 5, 8 and 12 with LOD scores >1.0. A maximum multipoint LOD score of 2.4 was obtained on chromosome 12. No linkage was found on chromosome 6p, or at the PAX2 locus on chromosome 10, both of which have been previously suggested might have a role in VUR. Further analysis of each of these chromosomal regions in 15 smaller New Zealand families with VUR failed to add significantly to the LOD scores. These data suggest that either VUR is caused by several genes in the population, or that there are several loci of major effect contributing to the disease.

Program Nr: 1504 from the 1999 ASHG Annual Meeting

High-throughput sequencing of candidate genes for alcohol-related QTLs. *M.A. Ehringer[1], J. Thompson[1], O. Conroy[1], Y. Xu[1], F. Yang[1], J. Canniff[1], M. Beeson[2], L. Gordon[2], B. Bennett[2], T.E. Johnson[2], J.M. Sikela[1]*. 1) Dept Pharmacology, Univ Colorado Health Sci Ctr, Denver, CO; 2) Institute for Behavioral Genetics, Univ Colorado, Boulder, CO.

We have developed a high-throughput automated gene sequencing approach to examine candidate genes within QTLs (Quantitative Trait Loci) related to alcohol action. Our efforts to date have focused on the four QTLs associated with inbred lines of mice which differ in their initial sensitivity to alcohol, inbred short-sleep (ISS) and inbred long-sleep (ILS). Using the rapidly evolving human and mouse gene maps, we have identified genes that fall within these QTLs and ranked them as possible candidate genes based on several criteria. Brain RNA isolated from appropriate animals is used to make cDNA, which is amplified using overlapping pairs of PCR primers specific for the coding regions of candidate genes. DNA sequences of PCR products are determined using ABI fluorescent automated sequencers, and the data is analyzed using a UNIX sequencing software package which includes Phred, Phrap, and Consed. Direct comparison of sequences obtained for each animal allows us to immediately determine if a DNA difference corresponds to a change in an amino acid. Thus far, a primary search of the four Lores has been completed. For example, approximately 123 genes within Lore2 have been evaluated and ranked using the latest data from the human gene map. Presently, the coding regions of eighty candidate genes have been comparatively sequenced and analyzed, either in whole or in part. We have identified five genes with DNA changes corresponding to amino acid differences between ISS and ILS mice, representing all four QTLs. This work is supported by grants AA11853 and AA03527.

Program Nr: 1505 from the 1999 ASHG Annual Meeting

Fragile X Mental Retardation in Syndrome in Saudi Arabia. *M.F. El-Hazmi.* Med Biochem, King Khalid Hosp, King Saud Univ, Riyadh, Saudi Arabia.

Mental retardation is a cognitive disorder frequently associated with various forms of disability. In a National survey, we identified 298 cases of mental retardation among 5,000 subjects, where mental retardation accounted for 10% of the disabilities. The aetiology of mental retardation is complex and several monogenic, polygenic and chromosomal abnormalities play a role in the development of mental retardation. One of the genetic causative factors of mental retardation is fragile X syndrome, a condition related to increased number of a polymorphic triple repeat sequence, (CGG), at a folate-sensitive fragile site, Xq27.3 (FRAXA). The number of the repeat sequence, located at the 5'-untranslated regions of FMR-1, is related to the mental disability. Males, due to their hemizygous state of X-chromosome, suffer more frequently from a mentally sub-normal state in patients with the number of repeats ranging from 54 to 200 (premutation), and mental retardation in patients with an increase in the repeat number more than 200. Normal men carrying a premutated allele are called `normal transmitting males (NTM) and have a high risk of having affected grandsons. Some affected individuals have premutations and full mutations and present mosaicism. A sample of forty two Saudi patients diagnosed as mentally retarded, and 800 cases with other disabilities were investigated using PCR amplification of DNA with primers flanking the trinucleotide repeats. Of the 42 mentally retarded group, 15 cases of full mutation and 8 cases of premutation were identified. The rest had normal PCR product pattern ruling out fragile X syndrome. Premutation pattern was also identified in the group with other disabilities including speech defects, learning disabilities. This paper will present an overview of diagnostic value of fragile X-syndrome in mental retardation, speech and learning disability.

Program Nr: 1506 from the 1999 ASHG Annual Meeting

Gene Dose Effect in Psoriatics Homozygous for HLA-Cw6. *C. Enerback[1], F. Enlund[2], A. Inerot[1], L. Samuelsson[2], J. Wahlstrom[2], G. Swanbeck[1], T. Martinsson[2].* 1) Dept Dermatology, Univ Gothenburg, Gothenburg, Sweden; 2) Dept Clin Genet, Univ Gothenburg, Sahlgrenska Univ Hosp, Gothenburg, Sweden.

Psoriasis is a common inflammatory skin disease with a heterogenous genetic background. The existence of a gene in the HLA region on 6p was suggested from association studies which in recent years has been confirmed by linkage studies. The purpose of this study was to study the Cw6 allele and its contribution to disease susceptibility in a patient material consisting of 104 families with at least two affected siblings. We have set up a sequencing method to examine the two exons that build up the antigen binding site of the C-locus receptor. Patients homozygous for Cw6 based on haplotype information was sequenced which confirmed the identity of the Cw6 allele in an affected individual with the consensus sequence. We screened the material for psoriasis patients homozygous for Cw6. Eleven individuals with a mean age at onset of 16,1 years was found, that in comparison with the mean age of 18,45 for the Cw6 heterozygotes and 22.36 for Cw6 negatives, points towards a gene dose effect. The method was also used to decide the genotype for the parents in order to perform a Transmission Disequilibrium Test on the Cw6 allele per se, used as a biallelic marker. This analysis resulted in a C^2 of 70,21 ($p= 5x10^{-17}$). This widely exceeds our previous results of TDT in this region, including microsatellite markers and SNPs in the coding part of the S gene (corneodesmosin). Also the maximum NPL value is reached using Cw6 as a marker. We conclude that Cw6 is the allele which show the highest degree of association to psoriasis in our material and we propose that it influences directly the age at onset of the disease rather than increasing the genetic load in accordance with a polygenic theory.

Program Nr: 1507 from the 1999 ASHG Annual Meeting

The alpha-synuclein-associated protein, synphilin-1: Gene structure and localization, and presence of synphilin-1 protein in Lewy bodies. *S. Engelender[1], K. Wakabayashi[2], T. Wanner[1], Z. Kaminsky[1], J.J. Kleiderlein[1], R.L. Margolis[1], S. Tsuji[2], H. Takahashi[2], C.A. Ross[1]*. 1) Dept Psychiatry, Johns Hopkins Univ, Baltimore, MD; 2) Brain Research Institute, Niigata University, Japan.

Parkinsons disease (PD) is a common neurodegenerative disease characterized by motor signs including tremor, bradykinesia and rigidity. Pathologically, there is loss of neurons in substantia nigra and the surviving neurons develop cytoplasmic inclusions termed Lewy bodies. Alpha-synuclein mutations cause some familial forms of PD, and alpha-synuclein protein is also present in Lewy bodies of patients with sporadic disease. Alpha-synuclein interacts *in vivo*with a novel protein we have called synphilin-1 (Engelender et al., Nature Genet. 22: 110-114, 1999). When synphilin-1 was co-transfected into mammalian cells with the NAC portion of alpha-synuclein we observed the formation of eosinophilic inclusions resembling Lewy bodies. We now find that synphilin-1 is present in some Lewy bodies of patients with PD and DLB. The staining of Lewy bodies with synphilin-1 is specific, since no staining is observed when the antibody is pre-absorbed with recombinant synphilin-1. We now have also cloned the human synphilin-1 gene, determined the organization of its10 exons and localized it to chromosome 5q23.1-23.3. Mutation analysis of synphilin-1 may further clarify the role of synphilin-1 in the pathogenesis of genetic PD. Furthermore, in sporadic PD, synphilin-1 could modulate alpha-synuclein aggregation.

Program Nr: 1508 from the 1999 ASHG Annual Meeting

Expression of the myotonic dystrophy locus genes in neonatal and adult tissues, and during muscle regeneration - indicators of possible pathological mechanisms. *M. Eriksson[1,2], T. Ansved[1], L. Edström[1], D. Wells[3], D. Watt[3], M. Anvret[1], N. Carey[4].* 1) Dept Neurology, Clinical Neuroscience, Stockholm, Sweden; 2) Dept Mol Med, Clin genetics unit, Karolinska Hospital, Stockholm, Sweden; 3) Division of Neurosciences,Imperial College School of Medicine,London,UK; 4) Division of Surgery, Imperial College School of Medicine,London,UK.

Myotonic dystrophy (DM) is a multisystemic disorder caused by the expansion of a CTG trinucleotide repeat, but the mechanism(s)by which this repeat leads to the very complex symptomatology remains controversial. One hypothesis is that the expansion disrupts the expression of the genes in its immediate vicinity - *DMPK*, *DMWD* and *Six5*. The pathological consequences of this phenomenon may vary between tissues, depending on the normal expression patterns of the DM locus genes. However, there have been no systematic, genuinely quantitative studies addressing the issue of relative expression levels of the genes at different time points in clinically significant tissues. It is also possible that the normal expression of the genes may be differentially regulated depending upon the physiological status of certain tissues. For example, we have already published data showing that *DMPK* expression increases in the muscles of patients with non-DM myopathies,and we hypothesise that this may be a consequence of increased muscle regeneration. We are using murine model systems to address these issues. Using highly sensitive and reproducible real-time fluorescence RT-PCR technologies the precise expression levels of the DM locus genes (relative to a house-keeping standard, TATA-binding protein) have been analysed in clinically relevant tissues from neonatal and adult C57Bl/10 mice. An *in vivo* system for stimulating skeletal muscle regeneration in the mice has also been employed for assessment of the effects of this process on expression of the genes. Our data provide essential baseline information on the expression of the DM locus genes, which is vital for selecting candidates for specific aspects of the pathology in this disease.

Program Nr: 1509 from the 1999 ASHG Annual Meeting

mtDNA mutation A1555G and progressive deafness: modifying factors of a common mutation causing late onset deafness. *X. Estivill[1], N. López-Bigas[1], R. Rabionet[1], O. Bravo[2], J. Girons[2], M.L. Arbonés[1].* 1) Medical & Molecular Genetics Center, Barcelona, Spain; 2) Otolaryngology Service, Bellvitge Hospital, Barcelona, Catalonia, Spain.

Late onset deafness is likely the result of complex interactions between genetic susceptibility factors and the environment. The A1555G mutation in the mitochondrial 12S rRNA has been associated with aminoglycoside-induced and non-syndromic sensorineural deafness. We have previously described 19 families with maternally transmitted deafness that have the A1555G mutation and we have analyzed the relationship of this mutation with the treatment with aminoglycosides. We have identified 66 new families with sensorineural hearing loss and have tested them for mutation A1555G. We have found the A1555G mutation in 11 of these families. By combining the families presented here with those described previously, we have identified 30 families with the A1555G mutation, representing 22% of all the deafness families that we have studied. Considering the 30 families with mutation A1555G, deafness was diagnosed in 214 of 429 subjects with the mutation, with a penetrance for A1555G of 50% by the age of 30 years and 90% by the age of 65. Interestingly, only 46 of 230 (20%) subjects with deafness of the 30 families with A1555G were treated with aminoglycosides prior to their hearing loss, indicating the strong role of factors as yet unknown in the development of deafness. This further confirms that the A1555G mutation is a major susceptibility factor for progressive deafness and that its penetrance can be enhanced by treatment with aminoglycosides. The identification of other factors involved in deafness due to A1555G should facilitate the understanding of the complex interactions between environmental and genetic factors in hearing loss. We are currently studying the relationship between A1555G and other deafness mutations in the mtDNA, specially mutation A7445G affecting the tRNA Ser(UCN), and mutations in the connexin 26 gene, for which a high carrier frequency have been described in the Mediterranean population. Mutations in these genes may enhance the development of deafness in patients that carry A1555G.

Program Nr: 1510 from the 1999 ASHG Annual Meeting

Search for imprinting effects in the hereditary paraganglioma critical region on chromosome band 11q23: allelic expression analysis of *PPP2R1B, POU2AF1,* D11S966E and methylation analysis of two *Not*I sites associated with novel genes. *J.E. Farr[1], J.R. Goss[2], P. Taschner[3], P. Devilee[3], U. Surti[4], B. Devlin[1], B.E. Baysal[1].* 1) Dept Psychiatry, Univ Pittsburgh Med. Ctr., Pittsburgh, PA; 2) Dept Neurology, Univ Pittsburgh Med. Ctr., Pittsburgh, PA; 3) Dept of Human Genetics Leiden Univ Med Ctr., The Netherlands; 4) Dept of Genetics, Magee Women's Hospital,Pittsburgh, PA.

Pedigrees with hereditary non-chromaffin paragangliomas (PGL, glomus tumors, MIM#168000) display features consistent with genomic imprinting: disease transmission through affected mothers has yet to be demonstrated whereas the disease phenotype segregates as autosomal dominant when transmitted through fathers. In accord genomic with imprinting, allelic imbalance patterns in PGL tumors display strong bias for the loss of normal, maternal allele. However, there are no other parent-of-origin effects described on chromosome band 11q23. *PGL1* has been confined to a 1.5 Mb critical interval. We evaluate allelic expression in three regional genes, *PPP2R1B* (encoding the beta isoform of PP2A-A subunit), *POU2AF1* (*Bob1*, *OBF1*, encoding Oct- binding factor 1) and D11S966E. For *PPP2R1B*, we obtain novel 3'-untranslated region sequence, which is compatible with the 5.0 kb transcript size in Northern analysis, and identify a new (CA/GT)n simple tandem repeat polymorphism (STRP) therein. Several fetal and adult tissues including brain, kidney, lung, chorionic villi, decidua and lymphoblastoid cell lines (LCL) show bi-allelic expression. For *POU2AF1* and D11S966E, we have tested an expressed (CCTT)n STRP and a single nucleotide polymorphism, respectively. Both genes show bi-allelic expression in LCL. We also capture two *Not*I sites associated with two novel genes in the *PGL1* critical region. Both *Not*I sites are completely de-methylated in LCL. Bi-allelic expression and de-methylation patterns are not altered in LCL from PGL affected and imprinted carrier individuals. The physical locations of the tested genes and *Not*I sites yield an even coverage of the critical region. These results provide the first direct evidence against domain-wide imprinting effects in the *PGL1* critical region.

Program Nr: 1511 from the 1999 ASHG Annual Meeting

A common in-frame deletion in the a_{2C}adrenergic receptor (a_{2C}AR): possible association with cocaine abuse. *J. Feng[1], J. Zheng[1], J. Gelernter[2], H. Kranzler[3], E. Cook[4], N. Craddock[5], D. Goldman[1], L.L. Heston[1], W.P. Bennett[1], S.S. Sommer[1]*. 1) Dept Molecular Genetics, City of Hope Natl Medical Ctr, Duarte, CA; 2) Dept Psychiatry, Yale University, West Haven, CT; 3) Dept Psychiatry, University Conn., Farmington, CN; 4) Dept Psychiatry, University Chicago, Chicago, IL; 5) Dept Psychiatry, Univ. Birmingham, Birmingham, UK.

All $a_2$adrenergic receptors (a_2ARs) are activated by adrenaline and noradrenaline, and three subtypes, termed A,B,C, are defined by differential affinities for antagonists including yohimbine and rauwolscine. All subtypes are expressed in major organ systems, and they bind common medications including antihypertensives, diuretics, antiemetics, sedatives, anxiolytics and neuroleptics. Compared to other subtypes, a_{2C}AR expression predominates in heart, lung, aorta, spleen, kidney, adrenal gland, cerebral cortex and cerebellum. Because the a_{2C}AR binds avidly to clozapine, which is used for treatment-resistant schizophrenia, we tested the hypothesis that a_{2C}AR polymorphisms predispose to schizophrenia or a behavior disorder. The coding regions of a_{2C}AR were scanned for polymorphisms using DOVAM-S in 100 schizophrenics and at least 25 patients in each of the following groups: autism, puerperal psychosis, alcoholism, cocaine abuse, and attention deficit/hyperactivity disorder. This analysis discovered three silent polymorphisms at codons 136, 148, and 388 (allele frequencies [AF] of 0.6-6.7%) plus a 12-base, in-frame deletion of codons 322-325 (Gly-Pro-Gly-Ala), which is the second of two homologous repeats. Del322-325 occurred commonly in African-American schizophrenics (11/28 subjects, AF 39%) and controls (AF 44%) and less commonly in 85 Caucasian schizophrenics of western European descent (AF 3.5%). Initial studies of behavior disorders suggest an association (p=0.029, Fisher's Exact Test) with Caucasian cocaine abusers (AF 8/80, 10%) compared to unrelated controls (AF 6/198, 3.0%). However, population stratification remains an issue and analyses continue. Since Del322-325 occurs within the third intracytoplasmic loop, it could plausibly alter the effects of therapeutic or recreational compounds.

Program Nr: 1512 from the 1999 ASHG Annual Meeting

The genetics of ischemic heart disease in the French-Canadian population: an association study. *C. Fournier[1], G. Turecki[1], C. Scali[1], C. Marineau[1], J. Grégoire[2], S. Bujold[2], P. Théroux[2], G.A. Rouleau[1]*. 1) Centre for Research in Neuroscience, McGill University and Montreal General Hospital Research Institute, Montreal, Canada; 2) Montreal Cardiology Institute, Montreal, Canada.

Cardiovascular diseases (CVD) are the leading cause of death in industrialized countries where 51% of deaths are related to ischemic heart disease (IHD). IHD is a complex disorder which is influenced by both environmental and genetic factors. As part of an ongoing study looking for genetic risk factors for IHD we conducted an association study in the French-Canadian population, where a significant founder effect has occurred. This population is especially well suited for the genetic study of complex traits due to a limited number of ancestral chromosomes resulting in decreased genetic heterogeneity. We studied 6 polymorphisms which have previously been implicated in the development of IHD: angiotensinogen (M235T), angiotensin-converting enzyme (287 bp I/D), plasminogen activator inhibitor-1 (4G/5G), apolipoprotein E, stromelysin-1 (5A/6A), and methylenetetrahydrofolate reductase (C677T). We investigated 165 French-Canadians with IHD aged 64.9+/-8.1 years (mean+/-SD) for the presence of the 6 genotypes listed above. The control group consisted of 85 French-Canadians, free of CVD, aged 64.0+/-7.6 years (mean+/-SD). We compared genotype frequencies between cases and controls using a chi-square test. There was no significant difference between cases and controls for all six genotypes. Moreover, no statistically significant differences were found when the analysis was repeated with the case and control groups segregated by gender. So far, the data from this study indicates that these polymorphisms do not contribute to the development of IHD in the French-Canadian population. A more stringent definition of the case and control groups as well as a more segregated analysis with respect to CVD risk factors might be needed to observe the possible effects of these genes.

Program Nr: 1513 from the 1999 ASHG Annual Meeting

Origin of Uniparental Disomy 15 in Prader-Willi and Angelman syndromes. *C. Fridman, C.P. Koiffmann.* Dept. of Biology, University of Sao Paulo, Sao Paulo, SP., Brazil.

Maternal uniparental disomy (UPD) accounts for ~25% of Prader-Willi syndrome (PWS) and paternal UPD for about 2-5% of Angelman syndrome (AS). These findings and the parental origin of deletions are evidences of genomic imprinting involvement in the PWS and AS aethiology. The PWS (neonatal hypotonia,hyperphagia,severe obesity,short stature,mental retardation)results from the loss of paternally expressed genes. The AS (ataxia, seizures, sleep disorder, hyperactivity, severe mental retardation with lack of speech, happy disposition) originates through the failure of maternally expressed gene(s). In a total of 34 PWS patients we disclosed 8 with maternal UPD and among 26 AS patients, 4 with paternal UPD. Analysing 10 microsatellite loci (CA repeats) spanning 15q (D15S541, D15S542, D15S11, D15S113, GABRB3, CYP19, D15S117, D15S131, D15S984, D15S115) we identified the meiotic origin of nondisjunction (ND) in all cases but two (1PWS and 1AS), and also the numbers of transitions (=observed changes in marker state between heterodisomy and isodisomy)in each case. A mitotic error was considered if all markers throughout the chromosome showed reduction to homozygosity. So, we disclosed 6 PWS heterodisomies originating from maternal meiosis I (MI) ND (3 showed heterodisomy throughout the entire chromosome with no evidence of recombination), 1 AS isodisomy (MII paternal error),3 isodisomies resulting from mitotic errors (1PWS,2AS). The maternal age was increased in our UPD samples; the mean age of PWS patients's mothers involving MI errors which showed no transitions from hetero to isodisomy was lower than of those showing one or more observable transitions (30,7 versus 41,5). These results confirm that the majority of maternal ND events resulting in UPD15 PWS are associated with MI errors, rare cases are MII or due to postzygotic errors, whereas most paternal UPD15 are MII or postzygotic events. The postzygotic errors are associated with an increased maternal age since the primary event is the maternal ND followed by fertilization of a nullosomic egg with a normal sperm and posterior duplication of the paternal chromosome in the zygote. Supported by: FAPESP.

Program Nr: 1514 from the 1999 ASHG Annual Meeting

Genome screening in human systemic lupus erythematosus: results from a second Minnesota cohort and combined analysis of 187 sib-pair families. *P.M. Gaffney[1], W.A. Ortmann[1], S.A. Selby[1], K.B. Shark[1], T.C. Ockenden[1], K.E. Rohlf[1], N. Walgrave[1], W. Boyum[1], M. Malmgren[1], G.M. Kearns[2], R.P. Messner[1], R.A. King[1], S.S. Rich[3], T.W. Behrens[1]*. 1) University of Minnesota Medical School, Minneapolis, MN; 2) University College, Dublin 7, Ireland; 3) Wake Forest University School of Medicine, Winston-Salem, NC.

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by a loss of immunologic tolerance to a multitude of self-antigens. Epidemiologic data suggests an important role for genes in the etiology of lupus, and previous genetic studies have implicated the HLA locus, complement genes and low affinity IgG (Fcg) receptors in SLE pathogenesis. In an effort to identify new susceptibility loci for SLE, we recently reported the results of a genome-wide microsatellite marker screen in 105 SLE sib-pair families (PNAS, 95:14875, 1998). We found strong evidence for linkage in four intervals: 6p11-21 (near the HLA), 16q13, 14q21-23, and 20p12.3, and weaker evidence (lod scores 1.0 and 2.6) in another 9 regions. We now report the results of a second complete genome screen in a new cohort of 82 SLE sib-pair families. In the Cohort 2 screen, the four best intervals were 7p22 (lod=2.87), 7q21 (lod=2.40), 10p13 (lod=2.24), and 7q36 (lod=2.17). Seven additional intervals were identified with lod scores ranging from 1.00 to 1.67. A combined analysis of MN Cohorts 1 and 2 (187 sib-pair families) showed that markers in 6p11-p21 (D6S426, lod=4.19) and 16q13 (D16S415, lod=3.85) met criteria for significant linkage. Three intervals (2p15, 1q42, and 7q36) have lod scores ranging from 1.92 to 2.06, and another thirteen intervals show lod scores ranging from 1.00 to 1.78 in the combined sample. These data, together with other available gene mapping results in SLE, are beginning to allow a prioritization of genomic intervals for gene discovery efforts in human SLE.

Program Nr: 1515 from the 1999 ASHG Annual Meeting

Mutation analysis of the a**7 nicotinic acetylcholine receptor gene and its partial duplication in schizophrenia patients.** *J.M. Gault[1], J. Logel[1,3], C. Drebing[1], R. Berger[1], J. Hopkins[3], A. Olincy[1], B. Sullivan[1], M. Short[3], K. Walton[1], M. Robinson[1], M. Maslak[3], J. Meriwether[3], L. Baldermann[1], S. Jacobs[3], R. Freedman[1,2,3], S. Leonard[1,2,3].* 1) Department of Psychiatry, Univ Colorado Health Sci Ctr, Denver, CO; 2) Department of Pharmacology, Univ Colorado Health Sci Ctr, Denver, CO; 3) Veterans Affairs Medical Center, Denver, CO.

Results from linkage analyses of pedigrees with schizophrenic members suggest that an auditory gating deficit and schizophrenia is linked to the 15q13-q14 region. The a7 neuronal nicotinic acetylcholine receptor (a7 nAChR) gene maps to the 15q13-q14 region and pharmacological investigation of the a7 receptor supports the hypothesis that it is involved in the auditory gating pathway that is disrupted in many schizophrenics. Exons 5-10 are proximally duplicated and are about 99% identical at the nucleotide level to the full-length a7 gene. Mutation analysis of both the a7 nAChR gene and its partial duplication in schizophrenics is presented. Southern blot, single strand conformation polymorphism (SSCP) and sequence analyses were used to identify variants in and flanking exons 1-10 of the full-length a7 gene. Four variants have been identified in or flanking exons 1-4. Eight variants have been identified in or flanking exons 5-10. Both genes are expressed with unique 5' ends allowing variants identified in exons 5-10 to be mapped to either the full-length a7 gene or its partial duplication by RT-PCR analysis using RNA isolated from either postmortem hippocampal brain or cycloheximide treated immortalized lymphocytes. Variants in the coding region that would obviously disrupt the function of the full-length a7 gene were not found and there is no evidence for gene conversion. Southern analysis using exon 5 as a probe detected 5 schizophrenics and one family member with the gating deficit that appear to be missing both copies of the duplicated a7 gene. The duplicated a7 gene message was not detected using RTPCR analysis of RNA isolated from three of these samples, supporting the hypothesis that these individuals are missing the duplicated a7 gene. The extent of the potential deletion of this region remains to be determined.

Program Nr: 1516 from the 1999 ASHG Annual Meeting

Identification of tumor necrosis factor receptor 2 (TNFRSF1B) as a novel mediator of insulin resistance traits in familial combined hyperlipidemia. *J.M.W. Geurts[1], C.J.H. van der Kallen[1], M.M.J. van Greevenbroek[1], R.G.J.H. Janssen[1], B.E. Aouizerat[2], H. Allayee[2], R.M. Cantor[3], A.J. Lusis[2], J.I. Rotter[3], T.W.A. de Bruin[1].* 1) Department of Internal Medicine, Maastricht University, the Netherlands; 2) Department of Microbiology and Molecular Genetics, University of California, Los Angeles, CA90095; 3) Department of Medicine and Human Genetics, University of California, Los Angeles, CA90095.

Familial combined hyperlipidemia (FCHL) is a common familial lipid disorder characterized by a variable pattern of elevated levels of plasma cholesterol and/or triglycerides, present in approximately 10 percent of patients with premature coronary artery disease. The genetic etiology of the disease is still largely unclear. Using a panel of Dutch FCHL families, a 10 cM genome screen was performed to identify chromosomal regions linked to genes contributing to this complex disorder. One of the loci identified by LOD score exhibited suggestive, but not significant, evidence for linkage to FCHL. After reviewing the genes in this region (chromosome 1p36.2), the TNFRSF1B gene was chosen as a possible candidate gene. A CA-polymorphism located within intron 4 of the TNFRSF1B gene was used as a marker to detect possible relationships between this gene and several clinical and biological variables related to the FCHL phenotype. Genotyping of this repeat in the families demonstrated the presence of 6 alleles (CA10, CA13, CA14, CA15, CA16 and CA17; of which 2 were hitherto unreported (CA10 and CA17). Alleles CA13, CA15 and CA16 were used for statistical analyses. Allelic distribution studies showed a significant enrichment of individuals homozygous for allele CA13 in the normoliplidemic relatives and a significant enrichment of individuals homozygous for allele CA15 in the hyperlipidemic subgroup. Furthermore, significant associations were demonstrated between these 3 alleles and several insulin resistance traits such as blood pressure, BMI, glucose, leptin, LDL-size, free fatty acids, soluble TNFRSF1B, waist and waist-hip ratio. Using this CA-repeat in additional two-point linkage analyses showed significant linkage to FCHL.

Program Nr: 1517 from the 1999 ASHG Annual Meeting

Normal human sperm exhibit greater mitochondrial DNA (mtDNA) heteroplasmy than ova. *C.D. Gocke[1], F.A. Benko[1], W. Dodson[2], P.K. Rogan[3]*. 1) Pathology, Penn State Coll Med, Hershey PA; 2) Obstetrics & Gynecology, Penn State Coll Med; 3) Medical Genetics & Mol Med, Children's Mercy Hospital, Kansas City, MO.

Background: We previously reported mtDNA heteroplasmy in 2.5% of normal mother-offspring pairs and marked genotype shifts, which were consistent with an estimated 252 to 505 mtDNA genomes segregating in oocytes. To test this, we assayed the D loop region of single oocytes for heteroplasmy, using single spermatids as controls. Materials and methods: Unfertilized oocytes discarded from IVF procedures on 5 women (12, 9, 7, 3, and 3 ova) were harvested, washed, lysed and amplified with D loop region primers. Donor sperm from 2 men (50 and 29 sperm) were counted by cytometry, diluted to a concentration of a single cell/tube, and similarly amplified. Lymphocyte DNA was available only from the women. Heteroplasmic variants were identified in PCR products by single strand conformational polymorphism analysis, sequenced and, if possible, confirmed by restriction digestion. Results: Three heteroplasmic loci were found in ova from 2 women. Spermatids had a higher frequency of heteroplasmy and polymorphism than oocytes. Polymorphisms were detected at 3 different loci in 29 sperm from one donor and at 19 loci in 50 sperm from the other. Conclusions: This study provides direct evidence of a bottleneck in mtDNA segregation during oogenesis. Genotype switching in oocytes was more frequent than we previously found in mother-offspring pairs, raising the possibility of either a post-meiotic bottleneck or of oocyte selection during IVF. Since identical variants are found in corresponding oocyte and lymphocyte mtDNA, heteroplasmy was inherited and not the result of mutations in ova. The inconsistent pattern of segregation of oocyte mtDNA may confound genetic counseling for mitochondrial disorders. By contrast, the presence of a variant in more than one sperm suggests that mutations arise early in spermatogenesis. The high frequency of homoplasmy for different variants in sperm from the same individual suggests that the population of segregating mtDNA species is smaller in sperm than in oocytes. This is consistent with a decreased population of mitochondrial genomes during sperm cell maturation.

Program Nr: 1518 from the 1999 ASHG Annual Meeting

Molecular genetic study of patients with hypertrophic cardiomyopathy. *M.V. Goloubenko[1], V.P. Puzgrev[1], K.V. Puzyrev[2], V.B. Salukov[1]*. 1) Population genetics laboratory, Institute of Medical Genetics, Tomsk, Russia; 2) Institute of Cardiology, Tomsk, Russia.

Idiopathic hypertrophic cardiomyopathy (HCM) is cardiac muscle disease caused in most cases by mutations in sarcomere proteins genes. Moreover, some mutations in mitochondrial DNA may also be cause for this disease. For investigation of genetic background of idiopathic HCM, the group of patients with HCM has been formed, consisting of 6 familial and 10 sporadic cases. Genomic DNA has been extracted to date from blood samples of 12 patients, as well as of some their relatives. Also, DNA will be obtained in near future from remaining 4 individuals and members of their families. Clinical assessment included medical history, echocardiography, and coronarography and allowed to exclude atherosclerosis and arterial hypertension as cause for hypertrophy. As the first stage of molecular genetic analysis, this sample was screened by RFLP analysis for the presence of A3243G mutation which is known as MELAS mutation but also may present in other diseases, including HCM. There were no patients who has this mutation, at least at the level detectable by routine electrophoresis. Further ascertainment of genetic basis of HCM in these patients will include sequencing of beta-myosine heavy chain gene which is responsible for about 50% cases of HCM.

Program Nr: 1519 from the 1999 ASHG Annual Meeting

Distance Between Marker and Trait-Affecting Loci in Linkage Disequilibrium Found in the General Population in Three Major Races: The Alzheimer's Disease, Hypertension, and Osteoporosis Genes. *G. Gong[1], J. Mordeson[2], S.-C. Cheng[2], N.C. Fong[2], R.R. Recker[1]*. 1) Osteoporosis Center, Creighton Univ, Omaha, NE; 2) Dept of Mathematics and Computer Science, Creighton Univ, Omaha, NE.

Alzheimer's disease (AD) is associated with the e4 allele at the apolipoprotein E (APOE) gene. It is possible that a mutation may have initially occurred somewhere close to, but not at, the APOE locus. The two loci may be in linkage disequilibrium, which is detected by association studies. The distance between the putative mutant and the APOE loci is mainly (under certain circumstances) a function of the degree of linkage disequilibrium and the number of generations past since the mutation. We developed a method to estimate the distance. The degree of disequilibrium was derived from published data. The age of the mutation was assumed to be 5000 generations. The assumption is based on the fact that the AD-e4 association is found in the general population in the three major races, which suggests that the putative linkage disequilibrium had existed before the divergence of the three races 100,000 years ago. The calculated distance was 35 ± 5 kb, which tends to be overestimated. Genetic heterogeneity and/or environmental factors (or their interactions) do not affect the estimation with our method. The two loci are so close that it is likely that the AD-predisposing mutation (or variation) falls within the span of the candidate APOE gene. The nature and the exact location(s) of the mutation(s) within the APOE gene still need to be determined. With a different approach, we also estimated the distance between marker (at the angiotensinogen gene and vitamin D receptor gene) and quantitative trait (blood pressure and bone mineral density) loci in association found in the general population in the three major races. The mean distances were less than 100 kb. We conclude that a well-established association between a marker and a trait (or disease) found in the general population in the three major races is likely to suggest that a mutation(s) is located within the span of the candidate gene, such as those in which certain polymorphisms are associated with AD, hypertension and osteoporosis.

Program Nr: 1520 from the 1999 ASHG Annual Meeting

Delineation of a 6p25 syndrome. *D.B. Gould[1], M.S. Jaafar[2], M.K. Addison[2], C.M. Powell[2], M.E. Walker[2], F.L. Munier[2], R. Ritch[2], I.M. MacDonald[1], M.A. Walter[1].* 1) Deptartment of Medical Genetics, University of Alberta, Edmonton, AB, Canada; 2) See poster for affiliated institution.

Our purpose is to further describe the phenotypes of patients with the 6p25 deletion syndrome and establish a genotype/phenotype correlation. Peripheral blood samples or cell lines were collected from patients with reported deletions of chromosome 6. PCR of polymorphic markers was used to establish copy number of alleles. FISH analysis has been initiated on available cell lines. Three patients have maximum deletions of 6.4cM, 27.2cM and 13.9cM. The fourth and fifth patients have a cytogenetic deletion at 6p25.1 and a ring(6) with breakpoints at 6p25q27 respectively, that have not yet been confirmed by molecular analysis. This could reflect a lack of markers in the region or the unreported presence of a translocation in the first case and mosaicism in the second. Three additional patients studied are deleted as the result of unbalanced translocations. Only one, a 2;6 translocation, has been confirmed by molecular analysis to have a maximum deletion of 6.4cM. The two remaining patients, with an unbalanced 6;8 translocation, and an unbalanced 4;6 translocation respectively, are currently being analyzed with polymorphic markers. Mutations resulting in haploinsufficiency of the FKHL7 gene at 6p25 have been shown to cause Axenfeld Rieger eye malformations. We suggest that haploinsufficiency of FKHL7 and other developmental control genes including FREAC2, BMP6 and AP2, also located at 6p24-6p25, are responsible for the 6p25 syndrome. The roles of these genes are under investigation. This work is supported by grants from the University of Alberta, Glaucoma Research Foundation, and the MRC.

Program Nr: 1521 from the 1999 ASHG Annual Meeting

TYPE I ALLERGY - A TOTAL GENOME SCANNING FOR CANDIDATE REGIONS AND TEST OF CANDIDATE GENES. *A. Haagerup[1], T. Bjerke[2], P.O. Schiøtz[4], R. Dahl[3], H.G. Binderup[1], T.A. Kruse[5]*. 1) Institute of Human Genetics, Aarhus University, Aarhus , Aarhus C, Denmark; 2) ASTRA Draco AB, Lund, Sweden; 3) Department of Respiratory Diseases, University Hospital of Aarhus, Aarhus, Denmark; 4) Department of Pediatrics, University Hospital of Aarhus, Aarhus, Denmark; 5) Clinical Institute, University of Odense, Odense, Denmark.

To identify genetic risk factors for atopic disease we collected 100 nuclear families containing at least two affected full siblings and both their parents. All family members, a total of 419 individuals, went through clinical investigation, questionnaire evaluation, and measurements of total and specific IgE. Two distinct strategies are used for DNA analysis: 1. A genome scanning with anonymous markers in an affected sib-pair design to identify candidate regions harbouring atopy candidate genes. 2. Test of specific candidate genes in a case-control design. 1. Genome scanning: Fifty families containing two sibs with type I allergy are being analysed with 500 micro satellite-markers evenly located through out the genome. The data is used for nonparametric linkage analysis. 2. Candidate genes: Variations in three candidate genes were analysed in all 100 families. A possible association between the variations and five different atopy phenotypes was tested using trio-families with internal controls. Il4R: We developed a simple PCR-based method to test genomic DNA for the Q576R polymorphism in the interleukine-4 receptor a subunit (chromosome 16p12). CCR-5: The delta 32 mutation in chemokine receptor five gene (3q21-24). CC16: The A38G variation in the Clara cell secretory protein (11q13). We did not find significant association between the tested genetic polymorphisms and type I allergy phenotypes.

Program Nr: 1522 from the 1999 ASHG Annual Meeting

Glucokinase gene is associated with Gestational Diabetes Mellitus in Chinese population. *H. Han[1], S. Wang[1], L. Ji[2], X. Han[2].* 1) Department of Obstetrics and Gynecology; 2) Department of Endocrinology and Metabolism, People's Hospital, Beijing Medical University, Beijing, P.R.China.

Gestational Diabetes Mellitus(GDM) is a status of impaired glucose tolerance during gestation and the individuals affected with GDM has a very high risk of developing Non-insulin dependent diabetes mellitus(NIDDM) later in their life. Early studies on glucokinase(GCK) gene had found several cases of NIDDM patients with history of GDM carried mutation in GCK gene. To test the hypothesis that the defect in GCK gene contribute to the genetic susceptibility to the GDM in Chinese population. We carried out a case-control study to investigate the allele distribution of two microsatellites, GCK1 and GCK2, in 40 women with history of GDM and 43 women without history of GDM and family history of NIDDM. Result: 1). 4 allele (A.B.C.D) of GCK1 and 4 alleles(1,2,3,4) of GCK2 were identified in both case and control groups. 2). There were not difference in the allele distribution of GCK1 in case and control groups. 3). The frequency of allele 3 of GCK2 was significantly increased in GDM group as comparing with that in the control group(31.3% vs 17.4%, P=0.035). 4). Further more, in GDM group, after stratifying 17 individuals who had developed NIDDM at time of this study by the alleles of GCK2, individuals carrying allele 3 (9 individuals) had a significant higher level of fasting plasma glucose and 2 hour plasma glucose in the OGTT test than that of those (7 individuals) carrying other alleles(9.9+ 3.7 mmol/l vs 6.3+ 0.6 mmol/l, p=0.019 and 15.9+ 4.4 mmol/l vs 11.2+ 2.2 mmol/l, p=0.020, respectively) . In addition, individuals carrying allele 3 had significantly decreased 2 and 3 hour insulin level in the OGTT test than that of those carrying other alleles (45.8+27.4 pmol/L vs 112.5+ 66.1 pmol/L, p=0.015 and 18.7+16.3 pmol/L vs 75.2+46.1 pmol/L, p=0.017, respectively). In conclusion, data from this study suggesting that the GCK gene is associated with GDM in Chinese population and allele 3 of GCK2 probably is in linkage disequilibrium with the genetic defects in GCK gene.

Program Nr: 1523 from the 1999 ASHG Annual Meeting

Methylation of the *UBE3A* CpG island does not mediate transcriptional repression of the paternal allele in brain.

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Angelman syndrome (AS) is a severe neurological phenotype involving profound mental retardation with absent speech, seizures, abnormal EEG, a movement disorder, happy disposition, and other features. AS results from deficiencies of imprinted gene expression of the E6-AP ubiquitin-protein ligase (*UBE3A*) gene, caused by common maternal deletion of human chromosome 15q11-q13, paternal uniparental disomy, imprinting defects, and discreet mutations in the *UBE3A* gene. Imprinted, maternal-specific expression of *UBE3A* and its mouse ortholog is restricted to the brain, with data from the mouse showing tight imprinted repression of the paternal allele in hippocampal and cerebellar Purkinje neurons. In peripheral tissues, such as leukocytes, *UBE3A* is expressed biallelically. DNA methylation is typically associated with transcriptional silencing of inactive alleles of imprinted genes; this includes the *SNRPN* gene, which is located within the adjacent, oppositely imprinted Prader-Willi syndrome critical region. *SNRPN* is associated with a 5' CpG island, which is heavily methylated on the transcriptionally silent maternal homolog. Unlike *UBE3A*, *SNRPN* is imprinted in all adult tissues, and displays allele-specific methylation accordingly. *UBE3A* is similarly associated with a 5' CpG island. To determine whether methylation of the *UBE3A* CpG island is associated with its brain-specific imprinting, we performed Southern blot analysis to test for methylation in brain and other tissues. Analysis of DNA from normal human hippocampus, cortex and leukocytes, using methylation-sensitive enzymes and probes spanning the CpG island, revealed no methylation in any of these tissue types. Similarly in mouse, analysis of DNA from tissue including hippocampus, cortex, and liver revealed no methylation of this CpG island, indicating that allele-specific methylation of this CpG island, in contrast to other CpG island-associated imprinted genes, such as *SNRPN* and *H19*, is not correlated with cell type-specific imprinting.

Program Nr: 1524 from the 1999 ASHG Annual Meeting

Gly40Ser Mutation of the Glucagon Receptor Gene is not a major determinant of genetic susceptibility to Non-insulin Diabetes Mellitus(NIDDM) in Chinese population. *X. Han, L. Ji.* Endocrinology and Metabolism, People's Hospital, Beijing Medical University, Beijing, P.R.China.

Recent studies had shown that a G123A mutation which causing a Gly40Ser substitution in exon 2 of the human glucagon receptor gene(GCG-R) was associated and linked with non-insulin-dependent diabetes mellitus (NIDDM) in France , Sardinia as well as Finnish population., but not with NIDDM in Japan , Russia as well as Utah of U.S.A, suggesting genetic heterogeneity of NIDDM in different ethnic groups. To investigate whether this mutation is contribute to genetic susceptibility to NIDDM in Chinese population, 150 NIDDM patients with family history of NIDDM were genotyped by using PCR-RFLP technique to screen for G 123 A mutation in GCG-R gene. As an result, none of the subjects studied showed this mutation. Our result suggested that Gly40Ser mutation of GCG-R gene is not associated with NIDDM in Chinese population. Thus, the Gly40Ser mutation does not play an important role in the pathogenethesis of NIDDM in Chinese population.

Program Nr: 1525 from the 1999 ASHG Annual Meeting

Maternal uniparental disomy of chromosome 7 (matUPD7) is confined to cases of Silver-Russel syndrome in children with growth retardation of unknown etiology. *K. Hannula[1], M. Lipsanen-Nyman[2], P. Höglund[1,3], C. Holmberg[2], J. Kere[1,4].* 1) Dept Medical Genetics, Univ Helsinki, Finland; 2) Hospital for Children and Adolescents, Helsinki Univ Central Hospital, Finland; 3) Jorvi Hospital, Dept Pediatrics, Espoo, Finland; 4) Finnish Genome Center, Univ Helsinki, Finland.

Maternal uniparental disomy of chromosome 7 (matUPD7) is observed in approximately 10% of Silver-Russel syndrome (SRS) patients. SRS patients are characterized by severe growth retardation and variable dysmorphic features, including hemihypertrophy, clinodactyly of the fifth digits, frontal bossing, and a triangular face. In order to ascertain if matUPD7 occurs in other growth-retarded patients in addition to SRS, we have studied DNA samples from 195 children with growth retardation of unknown etiology from the outpatient clinic for growth disorders at the Hospital for Children and Adolescents, Helsinki University Central Hospital. The patients formed three distinct groups by the following criteria: 1. Silver-Russel syndrome, 2. severe pre- and postnatal growth retardation exceeding -2.5 SD and 3. severe postnatal growth retardation exceeding -2.5 SD. Patients with endocrine abnormalities, abnormal karyotypes, chronic illnesses or any other known causes for growth retardation were excluded from study. MatUPD7 cases were screened for by genotyping DNA samples from each patient, mother and father with 14 chromosome 7 specific polymorphic microsatellite markers and an automated sequencer (ABI). Paternity for matUPD7 cases was confirmed by genotyping 12 non-chromosome 7 markers. Altogether four matUPD7 cases were observed among 33 SRS cases (12%). No matUPD7 cases were found in any other patient groups. The fact that matUPD7 cases are confined to patients with SRS strengthens the suggestion that multiple maternally imprinted genes may account for the various dysmorphic features in SRS patients that are not distinguished in other patient groups. It is nevertheless possible that disruption of imprinting of a gene/genes responsible solely for growth regulation would be the cause of growth retardation in other patient groups lacking dysmorphic features of SRS.

Program Nr: 1526 from the 1999 ASHG Annual Meeting

mRNA Differential Display Identifies Novel Strain Specific Ethanol Responsive (SSER) cDNAs Involved in Alcohol Preference. *M.L. Harrison, K.R. Uddin, J. Treadwell, B.C. Murphy, S.M. Singh.* Molecular Genetics Unit, University of Western Ontario, London, Ontario, Canada.

Alcohol preference is a well recognized contributor to the etiology of alcoholism, a complex disease known to run in families. A measure of alcohol preference is voluntary alcohol consumption (VAC), particularly in animal models (e.g. mice). Established genetic strains of mice are classified as alcohol preferer with high VACs (e.g. C57BL/6J) and avoiders with low VACs (e.g. A/J, DBA/2J and BALB/cJ). Despite extensive efforts over four decades, the search for VAC genes using traditional genetic approaches have not identified a single causitive gene. It is however, known that ethanol effects gene expression, possibly in a strain specific manner. Further such strain specific ethanol responsive (SSER) genes are expected to play a role in the determination of this complex phenotype. Also, it offers an opportunity to apply novel technologies such as mRNA differential display towards identification of SSER cDNAs.

Using the above approach, we have identified 10 brain specific SSER cDNAs. GenBank searches following sequencing of these bands permit one to classify them into two groups; a) no match in GenBank sequences and b) genes of known function. The sequences with no match in the GenBank database are of particular interest as they have the potential to represent still uncharacterized, novel genes. We are using GenomeWalker to obtain complete sequences towards the characterization of such genes and assess their significance in ethanol preference using segregation analysis including recombinant inbred (RI) lines. Identification and characterization of genes involved in murine alcohol preference will be a positive step in understanding the etiology of human alcoholism.

Program Nr: 1527 from the 1999 ASHG Annual Meeting

Single cell analysis of somatic mosaicism of CAG repeats in DRPLA brain. *H. Hashida[1,2], J. Goto[1,2], T. Suzuki[1], S.-Y. Jeong[1], N. Masuda[1,2], N. Hazeki[1], I.. Kanazawa[1,2].* 1) CREST, Japan Science and Technology Corporation, Tokyo, Japan; 2) Department of Neurology, University of Tokyo, Tokyo, Japan.

We report the analysis of somatic mosaicism of human triplet repeat gene using single cell isolated from human postmortem materials. We developed a system for RT-PCR analysis of single neuron isolated from autopsied human brain using laser microdissector. The single cells were dissected from unfixed brain specimen of the patients with dentatorubral-pallidoluysian atrophy (DRPLA) with laser microdissector after stained with toluidine blue. We demonstrate the transcripts of the normal and expanded alleles of CAG repeats by nested RT-PCR. Cell to cell difference was demonstrated in the length of CAG repeats of the expanded allele by the analysis of single cerebellar Purkinje cells isolated from autopsy materials of DRPLA.

Program Nr: 1528 from the 1999 ASHG Annual Meeting

Comprehensive Analysis of Gene Expression in Intracranial Aneurysm. *E. Heidrich-O'Hare[1], A. Kassam[2], H. Yonas[2], R. Ferrell[1], A. Brufsky[3], D. Peters[1].* 1) Human Genetics, University of Pittsburgh, Pittsburgh, PA; 2) Neurosurgery, University of Pittsburgh, Pittsburgh, PA; 3) Hematology/Oncology, University of Pittsburgh, PA.

Each year in the U.S., more than 25,000 people suffer a ruptured intracranial aneurysm (ICA). Among these, 50% die at the time of rupture or shortly thereafter and 25% suffer permanent disability, including paralysis, loss of speech, vision and motor coordination. The remaining 25% are at increased risk of stroke, recurrent bleeding or other complications. In contrast, operative repair of an unruptured aneurysm has a mortality of <2.5 % and morbidity of <6%. Despite their high frequency, the catastrophic consequences of a ruptured ICA and the difficulty and expense of operative surgical repair, almost nothing is known regarding the molecular pathology/pathogenesis of this disease. We have utilized a modified SAGE strategy ("SAGE-Lite") to compare gene expression patterns in a sample of unruptured ICA with a superficial temporal artery (STA) sample from the same individual, a two year old female who underwent elective craniotomy to resect multiple unruptured giant aneurysms in the middle cerebral artery. Analysis of the first 10,000 SAGE tags from each sample revealed marked differences at the level of transcription between normal and aneurysmal artery. Patterns of transcription are consistent with a pathological profile that involves extensive tissue remodeling and a significant inflammatory response. In summary, 31 % of the 100 most highly expressed genes in ICA are differentially regulated with a >5-fold change in expression relative to STA. Of these 100 genes, 22 % are not listed in GenBank. So far we have verified expression of 4,924 different genes in the ICA. Similarly, 29 % of the 100 most highly expressed genes in STA are differentially regulated with a >5-fold change in expression relative to ICA. Of these 100 genes, 25 % are not listed in GenBank. So far we have verified expression of 3,552 different genes in the STA.

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Program Nr: 1529 from the 1999 ASHG Annual Meeting

Mutations in the UBE3A gene are only a minor cause for Angelman syndrome (AS). *H.C. Hennies[1], J. Bürger[2], K. Sperling[2], A. Reis[1,2].* 1) Molecular Genetics, Max-Delbrueck Center, Berlin, Germany; 2) Human Genetics, Charité, HU Berlin, Germany.

Angelman syndrome (AS) is a severe neurogenetic disorder with a complex mode of inheritance. Clinically, AS is characterized by mental retardation, ataxia, and lack of speech. AS is caused by loss of function of an only maternally expressed gene. Around 70 % of AS patients show *de novo* maternal deletions at 15q11-q13, a few cases have a paternal UPD, and about 5 % have imprinting defects.

Mutations in the UBE3A gene, which encodes E6-AP and is paternally imprinted in brain, have been shown in non-deletion, non-UPD, non-imprinting-defect patients with AS. Until now, we have investigated 219 patients with a clinical diagnosis of AS for mutations in UBE3A by SSCP analysis of twelve translated exons representing the various open reading frames of the gene. Sequencing of conspicuous exons revealed eight different mutations in eight unrelated patients. All the mutations are insertions or deletions of two to 18 bases, five of these in-frame deletions and three frameshift mutations leading to premature stop codons. One two-residue deletion has been identified in three affected sisters and their unaffected mother, and one frameshift mutation is also of maternal origin. Six mutations are *de novo* ones. All alterations are compatible with paternal imprinting in AS and represent most presumably true mutations. Furthermore, five exonic polymorphisms were discovered in UBE3A.

Even considering that some of the patients might turn out to be misdiagnosed, our findings reveal a huge discrepancy between the number of mutations identified in UBE3A and the number of patients analyzed. In contrast to other studies with highly selected groups of patients, we can show that the disease is not due to mutations in UBE3A in the clear majority of non-deletion, non-UPD, non-imprinting-defect AS patients. Mutation analysis is still valuable in these patients for verification of the clinical diagnosis and genetic counseling. A further gene, however, or yet another mechanism must be conceived in the etiology of AS.

Program Nr: 1530 from the 1999 ASHG Annual Meeting

PARTICIPATION OF HLA DQA1 AND DQB1 LOCI IN THE INTRA-FAMILIAL HbA1 VARIATION OF FAMILIES WITH TYPE 1 DIABETES MELLITUS.. *R.M. Hermosillo-B[1], C. Ramos[2], F.J. Perea[1], F. Rivas[1], M. Casas-Catañeda[1], A.L. Camacho[1], Y.J. Sánchez[1], C. Gorodezky[3], B. Ibarra[1].* 1) Div. Genética, CIBO-IMSS, Guadalajara, Jal; 2) Hosp. Especialidades, CMNO-IMSS, Guadalajara, Jal; 3) INDRE-SSA, México, D.F. México.

In healthy individuals, variations in HbA1 level have been attributed to biological differences, or associated with several risk factors to develop diabetes. DQA1 and DQB1 genes are a major contributing risk factor in the pathogenesis of Type 1 Diabetes Mellitus (DM-1). Present work aims to analyze participation DQA1 and DQB1 alleles in the intrafamily variation of HbA1. We studied 20 families with DM-1 (115 individuals) and 19 control families (100 individuals). HbA1 values were analyzed among healthy members of DM-1 and control families to determine the intrafamily variation coefficient of HbA1 (ivc-HbA1). Molecular analysis of DQA1 and DQB1 alleles was performed by PCR-SSO and PCR-SSP. The average value of ivc-HbA1 was similar in both groups (11.98.0 and 9.84.5%). ivc-HbA1 of more than 10% was observed in 10 families of each group, whose average was similar except at ANOVA (17.66.9% and 14.03.3% respectively). Susceptibility (S) and Protection (P) DQA1 and DQB1alleles were analyzed in both groups considering independent chromosomes, parents of families with Normal ivc-HbA1 (less than 10%, DN and CN for parents of DM-1 and control families), as well as those with High ivc-HbA1 (DH and CH). Significant differences of allelic distribution were found for DQA1 and DQB1. For DQA1 differences were found among all groups except DH-CH and CH-CN. For DQB1 differences were found among all groups except DH-DN. The analysis of the total count of S and P alleles at both loci showed a main proportion of S alleles in the DH group, followed by DN, CH, and CN groups. We investigated the Relative Risk between all subgroups, the higher numbers were observed for DQA1 *0501 allele between DH-CN groups (7.18) and for DQB1 *0201 between DN-CH groups (7.22), The chi-square with Yates correction for both comparative groups was significant ($p< 0.02$ and 0.05 respectively). This data suggests the participation of both DQA1 and DQB1 loci at intrafamily variability of HbA1 levels.

Program Nr: 1531 from the 1999 ASHG Annual Meeting

A genealogical study of Parkinson's Disease in Iceland: Further evidence for a genetic component in late-onset disease. *A. Hicks[1], T. Jonsson[1], H. Petursson[1], S. Sveinbjornsdottir[2], J. Gulcher[1], K. Stefansson[1].* 1) DeCode Genetics, Reykjavik, Iceland; 2) National Hospital of Iceland, Reykjavik, Iceland.

Over recent years, the role of genetics in some forms of Parkinson's Disease (PD) has been clearly established. Mutations in the a-synuclein gene are involved in a small subset of autosomal dominant early-onset PD (< 50 years old), while mutations in the parkin gene have been identified as the primary cause of autosomal recessive juvenile parkinsonism. For the majority of late-onset PD (> 50 years old), both in kindreds and sporadic cases, mutations in these genes have been excluded. Furthermore, several cross-sectional twin studies have reported similar concordance between monozygotic (MZ) and dizygotic (DZ) twins, and have thus favored the influence of environmental factors over direct genetic effects. A recent longitudinal PET study of twins has reopened the debate with its findings of a strong genetic component in sporadic PD. To further examine the role of genetic components in the majority of PD cases, and to attempt their characterization, we are using a population-based genetic approach. Data from two epidemiological studies in Iceland, one in the 1960s and another from the 1980's onward, have succeeded in identifying over 750 PD patients born within the last 120 years (representing a large proportion of the total number of PD cases). We have compiled an extensive genealogical database of over 580 000 records dating back to the settlement of Iceland a thousand years ago. With this database we have used a number of statistical tools, including the newly developed Minimum Founder Test alongside kinship coefficient and relative risk calculations, to examine the relationships among these PD patients and to compare these with those among matched control groups in the general population. Our data demonstrate that in Iceland, there is a clear genetic component in all forms of PD examined.

Program Nr: 1532 from the 1999 ASHG Annual Meeting

***De novo* tandem duplication of 7p11.2-p12 in a patient with Silver-Russell syndrome and asynchronous replication timing further implicates *GRB10*as a candidate gene.** *M.P. Hitchins[1], D. Monk[1], E.L. Wakeling[1], V. Proud[2], P. Stanier[1], M.A. Preece[3], G.E. Moore[1].* 1) Paediatrics, Obstetrics & Gynaecology, Imperial College School of Medicine, Queen Charlotte and Chelsea Hospital, London, UK; 2) Daughters, 601 Childrens Lane, Norfolk, Virginia 23507-1921, USA; 3) Biochemistry, Endocrinology & Metabolism, Institute of Child Health, London, UK.

Silver-Russell syndrome (SRS) is a congenital disorder, characterised primarily by intrauterine growth restriction and lateral asymmetry. The aetiology of the syndrome is heterogeneous, but maternal uniparental disomy for chromosome 7 (mUPD7) has been demonstrated in 7% of patients. Consistent heterodisomy for the entire chromosome 7 in several mUPD7 cases provides strong evidence that one or more imprinted genes on chromosome 7 may be involved in the pathogenesis of this syndrome. Human 7p11.2-12 is syntenic to the imprinted region of proximal mouse chromosome 11, and mice with maternal disomy for this region demonstrate a growth-restricted phenotype.

We describe a *de novo*cytogenetic duplication of 7p11.2-12 in a SRS patient. Fluorescent *in situ* hybridisation (FISH) confirmed the presence of a tandem duplication encompassing the genes for the insulin-like growth factor binding proteins 1 and 3 (*IGFBP1* and *IGFBP3*), and the growth factor-receptor bound protein 10 gene (*GRB10*), but not the epidermal growth factor receptor gene. FISH analysis using PAC clones from 7p11.2-14 indicated that the duplication extends at least 8cM. The duplication was shown to have arisen on the maternally derived homologue, through dosage analysis of microsatellite markers. Biallelic expression of *IGFBP1* and *IGFBP3* has been observed during fetal development, making their involvment in SRS unlikely. Murine *Grb10*, which maps to proximal 11, was identified as a maternally expressed imprinted gene. Human *GRB10* is the prime candidate for SRS. We have used interphase FISH to investigate the replication timing of the region surrounding *GRB10*. Asynchronous replication was observed, which is a hallmark of imprinted genes, suggesting human *GRB10* may also be subject to genomic imprinting.

Program Nr: 1533 from the 1999 ASHG Annual Meeting

Improved screening methods for the polyglutamine $(CAG)_n$ tract length of the human androgen receptor gene.

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The length of the polymorphic CAG (polyglutamine encoding) repeat in exon 1 of the human androgen receptor (AR) gene is inversely related to the degree of transcriptional activation by the AR. Individuals with X-linked spinal and bulbal muscular atrophy have 40 or more CAG repeats. Increased AR repeat length has been associated with decreased prostate cancer risk in men or increased breast cancer risk in women with disease-associated germline BRCA1 mutations. We describe improved methods of sample preparation, amplification and initial size screening of the AR gene CAG repeat. The sizing may be performed nonradioactively and without fluorescent labeling using standard molecular biology laboratory equipment. DNA samples from noninvasive buccal (cheek) swabs can be used for PCR amplification of repeats with high G+C contents. In previous studies, amplification of the AR gene CAG repeat was inconsistent or required two rounds of PCR amplification. Detection often required radiolabeled PCR primers and/or extraction of the PCR product from an agarose gel prior to sequencing. We amplified buccal cell DNA under conditions optimized for the AR gene amplicon. Unlabeled PCR products were digested with restriction endonuclease PstI that generated a constant internal reference DNA fragment and a CAG repeat-containing fragment of variable length. The size of the repeat fragment in relation to the internal reference fragment was used to estimate the length of the short tandem repeat (STR). Samples containing repeats near the mean length of (CAG)21 can be easily recognized. Sequence analysis of selected amplicons showed that the gel migration of the constant fragment was slightly faster than the median length STR fragment, and that STR fragments differing by three nucleotides may be separated using a minigel apparatus. Amplicons containing STR's differing significantly from the median length can be selected for sequencing or more accurate sizing, avoiding the use of these costly procedures for all samples. The improvements in the process of G+C-rich repeat screening are applicable not only to the AR gene but also to a large number of STR amplicons.

Program Nr: 1534 from the 1999 ASHG Annual Meeting

Significant association of a single nucleotide polymorphism in the tumor necrosis factor-alpha gene promoter with human narcolepsy. *H. Hohjoh[1], T. Nakayama[1], J. Ohashi[1], T. Miyagawa[1], H. Tanaka[3], T. Akaza[3], Y. Honda[2], T. Juji[3], K. Tokunaga[1]*. 1) Human Gen,Sch Internatl Health, Univ Tokyo, Tokyo, Japan; 2) Seiwa Hospital, Tokyo, Japan; 3) Japanese Red Cross Central Blood Center, Tokyo, Japan.

Narcolepsy is a sleep disorder in which multiple factors including environmental and genetic factors are involved. A genetic factor strongly associated with the disorder has been found in the human leukocyte antigen (HLA) region: the DRB1*1501-DQB1*0602 haplotype predisposes to narcolepsy. No susceptibility genes other than the HLA-haplotype have been found. In this study, we performed an association study of tumor necrosis factor-alpha (TNF-a) gene with human narcolepsy, in which we examined the known single-nucleotide polymorphisms (SNPs) in the promoter region in 49 Japanese narcoleptic patients, who were all positive for DRB1*1501, and 111 healthy controls. The results indicated that the frequency of the genotype at position -857 (-857SNP) was significantly different between the patients and controls, and the allele frequencies of -857SNP revealed that the frequency of -857T was significantly increased in the patients compared with that in the controls: P = 0.0068. In addition, haplotypes presumed from HLA-DRB1, -857SNP and HLA-B loci suggested that -857T was mainly associated with DRB1 alleles other than DRB1*1501. This led the interpretation that the significant increase in frequency of -857T in the patients was not caused by allelic association with DRB1*1501. Thus, it is conceivable that the TNF-a with -857T was associated with narcolepsy independently of the strong association of DRB1*1501 with the disorder. To further examine the relationship between -857T and DRB1*1501, we investigated several Japanese narcolepsy families. From the haplotype analyses of the family members, both cis- and trans-associations between them were observed. It might be that the -857T-DRB1*1501 haplotype confers a strong predisposition to narcolepsy, although it is a minor haplotype in the healthy population. Altogether, these observations lead us to propose that TNF-a could be a new susceptibility gene in human narcolepsy.

Program Nr: 1535 from the 1999 ASHG Annual Meeting

Studies of the CAG repeat in the spinocerebellar ataxia type 7 gene in Taiwan. *M. Hsieh[1], S.J. Lin[4], J.F. Chen[2], H.M. Lin[1], K.M. Hsiao[3], C. Li[1], S.Y. Li[3]*. 1) Institute of Medicine; 2) School of Medical Technology; 3) Department of Life Sciences, Chung Shan Medical & Dental College, Taichung; 4) Department of Pediatrics, National Cheng Kung Univ.,Tainan,Taiwan, R.O.C.

Spinocerebellar ataxia type 7 (SCA 7) is an autosomal dominant disorder characterized by neural loss, mainly in the cerebellum and regions of the brainstem and particularly, the inferior olivary complex. This neurodegeneration disease is associated with expansion of unstable CAG repeats within the 5'-translated region of the SCA7 gene, located on chromosome 3p. We conducted a local survey of the normal population and candidate patients to analyze the distribution of the CAG repeat units in the SCA 7 gene in Taiwan by using the radioactive genomic polymerase chain reaction (PCR). The CAG repeat number ranged from 6 to 17 in the normal population, with the more common being around 8-13 repeats. The range is relatively narrow compared to that reported for other ethnic groups (7 to 35 CAGs). Meanwhile, by the use of a combination of PCR and southern blot analysis, one SCA7 family was identified and reported in this study. A marked instability of the CAG repeat number during transmission from father to son (41 vs. 100) was observed in the SCA7 family. Clinical anticipation is also significant in this family including an infantile case, whose age of disease onset is one month old. Up to date, the SCA7 mutation was detected in one out of 73 families with ADCA phenotypes, which is about 1.4 % of the ataxia families referred to us, compared to SCA1 (1.4%), SCA2 (9.6%) and SCA3/ MJD (27.3%) in our collection. In summary, we demonstrated that the PCR-based Southern blot analysis, with the advantages of sensitivity of PCR and specificity of Southern blot, is the most reliable diagnosis method for the SCA7 mutation screening. The molecular analysis technique makes possible the quick and accurate diagnosis of SCA7 patients and hopefully, in the future, applies to prenatal screening for SCA 7 families.

Program Nr: 1536 from the 1999 ASHG Annual Meeting

Fragile site at the myotonic dystrophy locus. *H.E. Hughes[1], E.E. Newman[2], J. Myring[1], P. Thompson[1], S.H. Roberts[1], E.A. Pickering[1], J.D. Brook[2]*. 1) Inst Medical Genetics, Univ Hosp of Wales, Cardiff, Wales; 2) Dept of Genetics, Queen's Medical Centre, University of Nottingham, Nottingham, UK.

Myotonic dystrophy is a progressive neuromuscular disorder caused by expansion of a CTG trinucleotide repeat within the 3untranslated region of the protein kinase gene, DMPK. We present the first known example of a fragile site at the DM locus in a patient with an expanded CTG repeat. The 10 year old female proband was investigated because of learning difficulties and confirmed myotonic dystrophy in her mother and other maternal relatives. Haplotype analysis on maternal relatives was carried out and the affected chromosome traced through the pedigree. DNA of the patient did not show an expanded CTG repeat on the maternal high risk chromosome that she had inherited. This raised the possibility that there had been a region of DNA deleted from the maternal chromosome or that an inter-allelic gene conversion event had occurred resulting in the apparent duplication of the paternal chromosome. Analysis using additional markers within the DM locus failed to resolve the situation as the patient was either heterozygous or uninformative for each of these markers. However, a Southern blot analysis performed on the DNA obtained from a lymphoblastoid cell line showed the presence of a paternal allele together with the maternally-derived expanded allele. Cytogenetic investigation of peripheral blood showed a fragile site at 19q13.32/3 in 12.7%. FISH studies with the probe F18894 showed that the fragile site mapped to the DM locus. Neither parental karyotype demonstrated a fragile site and the position of the chromosome 19 centromeric heterochromatin indicated that the chromosome containing the fragile site was maternally inherited. It is not clear why the original Southern blot failed to demonstrate the expansion in blood and if this failure can be directly related to the presence of the fragile site within the DM gene. It is has not been possible to test other tissues such as muscle. The increased sensitivity of the test in the lymphoblastoid cell line could be related to a number of enriched sub-populations of lymphocytes leading to an enhanced chance of detection.

Program Nr: 1537 from the 1999 ASHG Annual Meeting

Changes in the levels of heteroplasmy in cultured LHON cells. *K. Huoponen[1,2], V. Juvonen[1], E. Nikoskelainen[3], M. Penttinen[4], M-L. Savontaus[1,2].* 1) Medical Genetics; 2) Biology; 3) Ophthalmology; 4) Clinical Genetics Unit, University of Turku, Finland.

Heteroplasmy, the coexistence of wild type and mutant mtDNA in an individual, has been detected in about 14% of LHON families with the primary mutations. At present we have only a limited amount of knowledge of the mechanisms involved in segregation and distribution of heteroplasmic mtDNA. This poses problems in understanding the etiopathogenesis of the disease and genetic counselling of the heteroplasmic families since the transmission of the mutant mtDNA cannot be predicted. In order to study the behaviour of heteroplasmic LHON cells in long-term cultures, we established EVB transformed lymphoblastoid cell lines from five LHON patients having the ND4/11778 mutation. The percentage of wild type mtDNA in patients' blood was 6.2%, 11.4%, 36.0%, 62.5% and 65.8%, respectively. Using an accurate and sensitive minisequencing method, we quantified the levels of heteroplasmy in cell cultures at several points in time during 200-300 days. Our studies suggest that the initial proportions of wild type and mutant mtDNAs influence on which mtDNA type becomes ultimately fixed. If the proportion of one mtDNA type, mutant or wild type, is high enough, it eventually becomes fixed, and the other type is eliminated. The cells with 6.2% of wild type mtDNA became homoplasmic for mutant mtDNA, and the cells with 62.5% and 65.8% of wild type mtDNA shifted towards fixation of wild type mtDNA. There seemed to be a transition zone where either of the mtDNA types could become fixed or increase its proportion, as the behaviour of the cells with 11.4% of wild type mtDNA demonstrated: one cell line reached homoplasmy for the mutant mtDNA, while in the other cell line the amount of mutant mtDNA increased but did not reach homoplasmy. In the cells with 36% of wild type mtDNA the changes in the levels of heteroplasmy were slow, although generally towards increasing the amount of wild type mtDNA. Our observations suggest that neither of the mtDNA types have a replicative advantage, but the changes can be explained by non selective replication of both types of mtDNA molecules.

Program Nr: 1538 from the 1999 ASHG Annual Meeting

Mitochondrial DNA mutations in the tRNA$^{Ser(UCN)}$ gene causing maternally inherited hearing impairment. *T.P. Hutchin[1], M.J. Parker[3,4], I.D. Young[4], A. Davis[3], R.F. Mueller[2].* 1) Molecular Medicine Unit, St James's University Hospital, Leeds, U.K; 2) Department of Clinical Genetics, St James's University Hospital, Leeds, U.K; 3) MRC Institute of Hearing Research, University Park, Nottingham, U.K; 4) Department of Clinical Genetics, City Hospital, Nottingham, U.K.

Sensorineural hearing loss is the most common feature of mitochondrial diseases, often occurring in the presence of other clinical features. To date three point mutations in the mtDNA have been reported in cases of non-syndromic maternally inherited hearing impairment (MIHI); A7445G and 7472insC, both in the tRNASer(UCN) gene and A1555G in the 12S rRNA gene which also causes hypersensitivity to aminoglycoside antibiotics.

We describe two families from the U.K. with non-syndromic MIHI, one with the A7445G mutation and the other with a novel T to C mutation at position 7510, also in the tRNASer(UCN) gene. The T7510C mutation, which creates a Hin fI site, was not present in over 660 controls and will disrupt a highly conserved base pair in the acceptor stem of the tRNA.

Both mutations, although homoplasmic affect only the inner ear in these two families. Hearing loss is typically progressive but varies in age of onset and severity, although almost all family members with either mutation are affected. That three of the four mtDNA mutations currently identified in non-syndromic hearing impairment are in the tRNASer(UCN) gene may be coincidental or indicate a common mechanism for the pathology of these mutations, perhaps related to the processing of the tRNASer(UCN) in the cochlea.

The increasing number of reports of families with hearing impairment such as those here, highlights the importance of mtDNA mutations as a cause of non-syndromic hearing loss. mtDNA mutations may be more common than previously thought in non-syndromic hearing loss and should be screened for in all families compatible with maternal inheritance of deafness.

Program Nr: 1539 from the 1999 ASHG Annual Meeting

Polymorphisms in the TNF gene cluster are not associated to asthma in a German/ Swedish study sample. *T. Immervoll, M. Wjst.* Institute of Epidemiology, GSF Research Center, Neuherberg, Germany.

Recently a genome wide screen in an affected sib pair study of a mixed German/ Swedish sample including 97 families and 156 sib pairs was finished. Microsatellite typing with a panel of 351 markers evenly spaced over the genome with about 10 cM distance yielded 4 chromosomal regions with suggestive linkage for asthma. Chromosome 6 around the marker D6S291 showed a p-value of 0.008 (Wjst et al., 1999) The surrounding region of this marker contains the asthma candidate genes tumour necrosis factor (TNF) and lymphotoxin (LT). Two already known single nucleotide polymorphism were screened. The first (LTaNcoI 1/*2) is located in the second intron of LT and creates a new NcoI site. The second (TNF-308*1/*2), which has been analyzed by sequence specific oligonucleotide (SSO) probing, is located in the promoter region of TNF. Both polymorphisms do not show significant association with asthma in the described population. Wjst, M., Fischer, G., Immervoll, T., et al., (1999), Genomics, 58:1-8. A genome wide search for linkage of asthma.

Program Nr: 1540 from the 1999 ASHG Annual Meeting

Abnormal expression of a**1A-voltage-dependent calcium channel protein in brains of spinocerebellar ataxia type 6 (SCA6).** *K. Ishikawa[1,3], H. Fujigasaki[1,2,3], H. Saegusa[2,3], N. Ohkoshi[4], S. Shoji[4], T. Tanabe[2,3], H. Mizusawa[1,3].* 1) Dept Neurology, Tokyo Medical & Dental Univ, Tokyo, Japan; 2) Dept Pharmacology, Tokyo Medical & Dental Univ. Tokyo, Japan; 3) CREST, Japan Science and Technology Corporation, Saitama, Japan; 4) Dept Neurology, Inst. Clinical Medicine, The Univ. of Tsukuba, Tsukuba, Ibaraki, Japan.

Spinocerebellar ataxia type 6 (SCA6) is one of at least eight neurodegenerative disorders caused by expansion of trinucleotide CAG repeat in the a1A-voltage-dependent calcium channel gene (CACNA1A). Neuropathology show predominant neuronal loss of the Purkinje cell in the cerebellar cortex. To clarify the pathophysiology in SCA6, we analyzed the expression of a1A-calcium channel protein in normal and SCA6 brains using two polyclonal antibodies raised against synthetic peptides corresponding two different portions of the channel protein. Of note is that one of these antibodies recognizes only the extended C-terminal region of the channel protein, containing polyglutamine tract. Immunohistochemically, a variety of neurons throughout the normal human brain were immunoreactive with both of the antibodies. However, the Purkinje cell showed the most intense immunoreaction, indicating that the channel protein is most abundantly expressed in this type of neuron. In SCA6 brains, aggregation of the channel protein was seen in the cytoplasm of Purkinje cells. This cytoplasmic inclusion was specific to SCA6 Purkinje cells, since similar inclusion was not seen in any other neurons of the SCA6 brains, nor in brains affected with other CAG repeat/polyglutamine diseases. In addition to the aggregation formation, reduced immunoreactivity against the calcium channel antibody was seen in the molecular layer and dentate nucleus, which may suggest altered distribution or reduced expression of the a1A-calcium channel protein in the cerebellum. There were no obvious changes in immunoreactivities in all other regions of the SCA6 brains. We conclude that formation of aggregation and altered expression of channel protein could be strongly related to the pathophysiology of SCA6.

Program Nr: 1541 from the 1999 ASHG Annual Meeting

The mitochondrial C3303T mutation is highly segregated in ragged-red fibers. *R. Iwanaga, Y. Koga, Y. Akita, S. Yano, T. Matsuishi, H. Kato.* Department of Pediatrics and Child Health, Kurume University School of Medicine.

A C3303T mutation in the mitochondrial tRNALeu(UUR) gene was reported as the family with maternal inherited cardiomyopathy. However, there is no described information about the relationship between pathological, biochemical, and genetical findings on this C3303T point mutation. In our case, we analyzed the percentage of this mutation in various autopsied tissues to evaluate the correlation between pathology and heteroplasmic condition, and also we performed the single fiber analysis by PCR/RFLP method to evaluate the distribution of this mutation in the single fiber tissue. Five months old Japanese girl was admitted to our hospital showing generalized muscle weakness, hepatomegaly, and cardiomegaly with lactic acidosis and died at six months of age. Skeletal muscle showed severe degenerating myopathy shown as full of ragged-red fibers, increased numbers of lipid droplets, and severe COX deficiency. Microscopically hepatocytes showed massive accumulation of lipid droplets and heart muscle showed network pattern suggesting metabolic cardiomyopathy. Activities of respiratory chain enzyme complex I plus IV in the skeletal were 23.4% and 5.0% of the control value, significantly decreased. The percentages of C3303T mutation in the patient tissues are quite variable, range from 25% in pancreas to 99% in spinal cord. By the single fiber analysis the percentage of C3303T mutation in RRFs with COX negative (Group1) (42.4+7.0) and in RRFs with COX positive (Group2)(58.2+5.8) were significantly higher than that in non RRFs with normal COX staining (Group3) (10.7+6.3) ($p>0.001$). Although the pathological abnormality in Group1 was much severe than in Group 2, the percentage of mutant DNA in Group 1 was significantly lower than in Group 2($p>0.05$). Our patient showed fatal infantile from of encephalopathy, myopathy and cardiomyopathy associated with widely distributed C3303T mutation in all of her somatic cells. Since her skeletal muscles showed severe pathological changes but the percentage of mutation in her muscle were only 67%, this mutation may have more potent biological abnormality than that in A3243G mutation.

Program Nr: 1542 from the 1999 ASHG Annual Meeting

Limited expression of SCA8 is consistent with cerebellar pathogenesis and toxic gain of function RNA model.
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It is intriguing to consider how untranslated CTG expansions cause diseases as distinct as spinocerebellar ataxia type 8 (SCA8) and chromosome 19 myotonic dystrophy (DM1). DM1 has been distinct among the triplet repeat disorders both in terms of the transcribed repeat motif (CTG) and its location in the 3' untranslated region of this gene. We recently isolated a second example of a pathogenic CTG expansion in SCA8. As in DM1, the SCA8 mutation is an untranslated CUG expansion at the 3' end of an RNA. This is the first dominant spinocerebellar ataxia not caused by an expanded CAG repeat translated as a polyglutamine tract. Affected individuals develop spastic and ataxic dysarthria and limb movements. However, systemic features of DM are absent in SCA8. There is no predilection for cardiac arrhythmias, cataracts, glucose intolerance, or gonadal failure, nor do SCA8 patients have clinical or electrical myotonia or myopathic weakness. The expanded CUG transcripts in SCA8 and DM1 might share a common toxic RNA mechanism at the cellular level. To evaluate if the SCA8 tissue distribution correlates with clinically affected tissues, Northern and multiple tissue blots were probed. Only weak hybridization signals were detected in various brain tissues. RT-PCR analysis confirmed that SCA8 is expressed primarily in the brain. SCA8 PCR analysis of normalized first-strand cDNA from mRNA extracted from eight human tissues was performed. PCR products were detected in brain and very weakly in lung but not in heart, placenta, liver, skeletal muscle, kidney and pancreas. Additional RT-PCR experiments detected transcripts in cerebellum but not in skeletal muscle and blood. The limited expression of SCA8 correlates with the cerebellar atrophy. We hypothesize that the disease mechanisms for SCA8 and DM1 are similar, and that the clinical differences between these diseases are due to the different expression profiles of the SCA8 and DM1 transcripts. We are testing this hypothesis by further characterizing SCA8 gene expression and by developing a mouse model of the disease (also see Koob et al.).

Program Nr: 1543 from the 1999 ASHG Annual Meeting

Paraoxonase May Contribute to Vascular Disease Through an Effect on LDL Density. *G.P. Jarvik, T.S. Hatsukami, A. Chait, C.E. Furlong.* Dept. Medicine, U. Washington, Seattle.

Paraoxonase (PON1) is a high density lipoprotein (HDL)-associated enzyme that has been suggested to protect low density lipoprotein (LDL) from oxidative modification. The $PON1_{Q/R192}$ polymorphism results in an amino acid 192 substitution (Gln/Arg). The R allele has been predictive of vascular disease (CVD) in several, but not all, studies and is carried by over half of Caucasians. We evaluated the relationship of PON1 and another atherogenic LDL feature, dense LDL, in 105 Caucasians with carotid artery disease (over 80 percent stenosis by angiogram). The subjects were US military Veterans, mean age 69 yr (range 49-83 yr); 103 were male, 67 hypertensive, 21 diabetic, 87 former and 39 current smokers. LDL flotation rate (LDL-Rf), a quantitative measure of LDL density, was determined using non-equilibrium density gradient ultracentrifugation.

$PON1_{192}$ genotype was predictive of LDL-Rf (p=.01), with the RR genotype being associated with denser LDL (LDL-Rf was not normally distributed, thus a Kruskal-Wallace Mean Rank test was used; mean ranks of QQ, QR, and RR genotype subjects were 55, 56, and 22; mean LDL-Rfs were .268, .265, and .228, respectively). Adjustment of LDL-Rf for LDL oxidative susceptibility using 3 measures of copper-mediated oxidation of LDL did not eliminate the significant $PON1_{192}$--LDL-Rf relationship. The $PON1_{55}$ polymorphism did not add additional information about LDL density once $PON1_{192}$ genotype is considered, but PON1 enzyme activity for the hydrolysis of paraoxon and diazoxon did add information about LDL density.

Thus, $PON1_{192}$ genotype predicts variation in LDL density in these vascular disease subjects. Of interest, this sample has multiple CVD risk factors and genetic factors may have been expected to play less of a role. Gene by environment interactions may exist. Although LDL density has been associated with LDL oxidative susceptibility, an independent PON1-LDL-Rf relationship is suggested by the significance of PON1 predictive power on oxidative status-adjusted LDL-Rf. This may represent a previously undescribed biologic mechanism for the reported role of the $PON1_{192}$ polymorphism in CVD.

Program Nr: 1544 from the 1999 ASHG Annual Meeting

Analysis of Affected Sibling Pairs with Rheumatoid Arthritis: The North American Rheumatoid Arthritis Consortium. *D. Jawaheer[1,4], T. Costello[2,4], C. Amos[2,4], J. Monteiro[1,4], M. Seldin[3,4], L. Criswell[4], SL. Bridges[4], H. Schroeder[4], D. Pisetsky[4], D. Kastner[4], R. Wilder[4], R. Pope[4], D. Clegg[4], R. Ward[4], S. Albani[4], JL. Nelson[4], M. Wener[4], L. Callahan[4], T. Pincus[4], PK. Gregersen[1,4].* 1) Dept Biol & Human Genetics, North Shore Univ Hosp, Manhasset, NY; 2) MD Anderson, Houston, TX; 3) UC Davis, Davis, CA; 4) The North American Rheumatoid Arthritis Consortium.

The North American Rheumatoid Arhtiris Consortium (NARAC) has, to date, identified 491 families with one or more sibling pairs affected with rheumatoid arthritis. At least one affected sibling in each family is required to have erosions on hand X-rays. Affected siblings have also been evaluated upon examination using a modified joint alignment and mobility score (JAMS). 77% of the affected siblings are female and 23% are male. The mean age of disease onset was 37 and 41 for females and males, respectively, with mean disease duration of 17(+/- 11.7) years. 82% of patients are RF+ and 92% have erosions in both hands. The mean JAM score is 27.8 (range 0-117). These data indicate that the selection criteria for NARAC have resulted in a collection of RA sibling pairs with relatively early onset, longstanding and severe disease. A genome-wide screen using microsatellite markers from the Marshfield set 8A is currently being performed on the first 158 families. In addition, 4 markers within the HLA region (D6S265, D6S1629, D6S273 and D6S291) have been used to evaluate whether linkage can be detected with this current data set. Analysis of the HLA data from 184 affected sib pairs, using Sibpal from the S.A.G.E ver 3.1 package, showed significant linkage with disease, especially for markers D6S265 and D6S273 (p=0.001). Haplotyping with GENEHUNTER of HLA loci led to an estimate of 15% of sib pairs sharing 0 alleles i.b.d. which yields a 1.7-fold relative risk estimate associated with HLA.

Program Nr: 1545 from the 1999 ASHG Annual Meeting

DRD2 not associated with obesity in Pima Indians. *C.P. Jenkinson, K. Cray, R. Hanson, C. Wiedrich, C. Bogardus, L. Baier.* PECRB, NIH/NIDDK, Phoenix, AZ.

The gene for the type 2 dopamine receptor, DRD2, has been associated with various addictive, impulsive disorders, including alcoholism, schizophrenia and obesity (Noble et al., Int. J. Eat. Disord. 15:205-217, 1994; Noble, Alcohol, 16:33-45, 1998). In an autosomal genomic scan for genetic markers linked to obesity and type 2 diabetes in Pima Indians, the region most strongly linked to obesity was on chromosome 11q22-24 (Hanson et al., Am. J. Hum Genet. 63:1130-1138, 1998; lod = 3.6 for BMI). This complex disease gene localization has subsequently been partially replicated in a Caucasian population (Jaquish et al., Am. J. Hum Genet. 63:A329, 1998; lod = 3.2 for BMI). The fact that DRD2 maps to this locus makes it a strong positional candidate for susceptibility to obesity. To further investigate this possibility we analyzed single nucleotide polymorphisms (SNPs) within the DRD2 gene for association with BMI. The SNPs (Ser311Cys, a C®G transversion and TaqAI, a T®C transition) were genotyped in the same group of 1200 subjects which had provided evidence for linkage in the Pima population. Ser311Cys is a coding sequence mutation which severely reduces dopamine signal transduction through DRD2. TaqAI, located in the 3 non-coding region, has provided evidence for association with obesity in other populations. All analyses were performed on the residual of the log-transformed maximal BMI, adjusting for age, sex, birth year, and nuclear family membership. Among full-heritage Pimas, individuals with a heterozygous CG genotype at Ser311Cys had a slightly higher BMI than those with CC homozygosity (36.7 vs. 35.5 kg/m2, p=0.03), although a test of the overall null hypothesis that all three genotypes are equal was not significant (p=0.08, 2 df). The T®C change at the TaqAI site was not significantly associated with BMI. Adjustment for these polymorphisms had no significant effect on linkage for BMI. Thus DRD2 does not appear to be the putative major gene for BMI on chromosome 11.

Program Nr: 1546 from the 1999 ASHG Annual Meeting

Nuclear receptor co-activator (ACTR), a candidate gene for type 2 diabetes identified by fine mapping of 20q13.1: results of mutation analysis. *L. Ji[1,2], T. Klupa[1,2], A. Antonellis[1], M. Malecki[1,2], J.H. Warram[1], A.S. Krolewski[1,2]*. 1) Dept Genetics & Epidemiology, Joslin Diabetes Ctr, Boston, MA; 2) Department of Medicine, Harvard Medical School.

Our study provided strong evidence for a novel susceptibility gene for type 2 diabetes located on 20q13.1, most likely in a critical region defined by markers D20s178 and D20s197. Several other family studies have also provided evidence pointing to these markers. To facilitate the identification and cloning of diabetes susceptibility genes in this region, we constructed a contig covering the chromosomal region that includes D20s178 and D20s197. We used STSes and ESTs on GeneMap '99 to construct the contig from four PAC sequences (Sanger Center) and 5 BAC clones identified either electronically or manually. In addition to 10 ESTs, one gene named nuclear receptor co-activator (ACTR) was mapped to the contig. ACTR is a histone acetyltransferase that plays a central role in DNA transcription by forming a multimeric activation complex with P/CAF and CBP/p300. Because of its presumed role in insulin gene transcription and the recent successes in identifying diabetes genes that are involved in insulin transcription, we were prompted to test whether a genetic defect in the ACTR gene might be responsible for type 2 diabetes that is linked with Chromosome 20. We screened the coding sequence of the ACTR gene in the probands of each of seven families with type 2 diabetes linked with 20q and found mutations in three of them. Two mutations did not segregate with diabetes while one, a missense mutation Tyr469Ser, segregated with type 2 diabetes in one family. A further search for mutations in the promoter region of the ACTR gene is being made in the 6 remaining families.

Program Nr: 1547 from the 1999 ASHG Annual Meeting

Kinetics of mitochondrial iron turnover in Friedreich ataxia cells. *S. Jiralerspong[1], L. Montermini[1], M. Pandolfo[1,2]*. 1) Dept. of Neurology, Centre Hospitalier de l'Université de Montréal, Montréal, Canada; 2) Dept. of Neurology and Neurosurgery, McGill Univ., Montréal, Canada.

Studies in yeast suggest that *YFH1*, the gene encoding a homologue for the Friedreich ataxia (FRDA) protein frataxin, is important for maintaining mitochondrial iron homeostasis. Moreover, human fibroblasts from FRDA patients show increases in steady-state mitochondrial iron levels by direct measurement. These increases have been fairly subtle and have failed to convincingly validate the yeast model for frataxin function as applied to mammalian systems, especially in light of the absence of iron accumulation in bacteria deficient for the frataxin homologue *CYAY*. Consequently, we hoped to clarify this issue and detail the dynamics of mitochondrial iron cycling by performing pulse-chase analysis on fibroblasts. We have observed significant differences in the kinetics of mitochondrial iron turnover between FRDA and control cells, even in cells with only marginal decreases in frataxin expression. Control cells preloaded with iron rapidly accumulate and export additional iron, while patient cells show steady, modest increases in iron over the same time period. Our results confirm that mitochondrial iron cycling is altered in FRDA cells as in yeast cells. Further studies to define the pathways directly influenced by loss of frataxin function are underway.

Program Nr: 1548 from the 1999 ASHG Annual Meeting

Multiple Autoimmune Disease Regulatory Loci Cluster on Rat Chromosome 10 and Homologous Mouse and Human Chromosomes. *B. Joe[1], E.F. Remmers[1], M.M. Griffiths[2], R.L. Wilder[1]*. 1) Arthritis and Rheumatism Branch, NIAMS,NIH,Bethesda,MD 20892; 2) Research Service Veterans Affairs Medical Center and Department of Medicine/Rheumatology, University of Utah, Salt Lake City, UT 84132.

Infection and immunity are biologically inter-linked processes. Dysfunctional regulation of immune responses to infectious agents may lead to autoimmune diseases. Among several factors that affect susceptibility to infectious diseases and autoimmune responses, genetic factors are being identified using animal models of infectious and autoimmune diseases, respectively. Evidence from several genetic mapping studies indicates that regulatory loci for susceptibility to infectious and autoimmune disease traits cluster on rat chromosome 10 and homologous regions on mouse and human chromosomes. We have identified two regulatory loci, Cia5 and Ciaa2, that regulate severity of collagen-induced arthritis (CIA) and levels of autoantibody production, on rat chromosome 10. Cia5 not only overlaps with regulatory loci for oil-induced arthritis (Oia2) and carrageenan induced inflammatory exudate volume in rats, but also with regulatory loci for leishmaniasis (Scl1) and autoantibody production in mice, and loci for psoriasis (PS) and multiple sclerosis (MS) in humans. Ciaa2 overlaps with Eae7, Idd4 and Orch3, which are loci on mouse chromosome 11 regulating experimental autoimmune encephalomyelitis (EAE), insulin dependent diabetes mellitus (IDDM) and orchitis, respectively and with MS in humans. In addition to Cia5 and Ciaa2, rat chromosome 10 additionally harbors a third cluster of loci that regulates susceptibility to experimental autoimmune uveitis (EAU) and EAE, onset of diabetes and levels of IgE. These loci include the IL-3, IL-4 and IL-5 regions at rat chromosome 10 (25-50cM). Homologous regions bear susceptibility loci for lupus linked traits (Lbw8, Nba), EAE (Eae6), S. typhi infections and IL-12 responsiveness (Tpm1) in mice and a regulatory locus for IgE levels in humans. Overlapping of regulatory loci on rat chromosome 10 and its homologous regions in other species suggests that rat chromosome 10 contains genes controlling susceptibility to infections and autoimmune responses.

Program Nr: 1549 from the 1999 ASHG Annual Meeting

The interaction of transcriptional regulatory proteins with huntingtin: implications for Huntington's disease aetiology. *L. Jones[1], P. Thomas[1], L. Elliston[1], P.S. Harper[1], J.W. Neal[2]*. 1) Institute of Medical Genetics and; 2) Dept of Pathology, University of Wales College of Medicine, Cardiff, Wales, UK.

Huntington's disease (HD) is an autosomal dominant neurodegeneration associated with an expanded CAG repeat at the 5' end of a large gene, which upon translation gives an expanded polyglutamine tract at the N-terminus of the protein, huntingtin. We previously isolated a transcriptional repressor protein, the nuclear receptor co-repressor (N-CoR) which interacts specifically with the polyglutamine-bearing N-terminus of huntingtin. The strength of interaction is modulated by repeat length; longer repeats have a stronger interaction with huntingtin. There appears to be no interaction with other proteins carrying polyglutamine repeats. We have followed up this finding in human HD and control postmortem brain tissue, examining the distribution of N-CoR and other proteins known to interact with it in the formation of repression complexes. N-CoR itself is detected in control brain, localised in the neuronal cytoplasm and also the nuclei of cortical neurons, particularly in layers V and VI. In HD brain cortical neurons, however, N-CoR immunoreactivity is localised in the cytoplasm and the nuclear localisation appears to be abolished. N-CoR immunoreactivity was not localised to the characteristic intranuclear inclusions of HD brain. However, other proteins which interact with N-CoR within the repression complex, are found to localise to the NII of HD brain. mSin3 and HDAC1 were both detected localised to a subset of inclusions in Grade III and IV HD brain. This study is currently being extended to Grade I and II HD brain. Further *in vitro* experiments have also indicated changes in the transcription of reporter genes in the presence of N-terminal huntingtin, implying that the repression complex may be recruited by huntingtin through its interaction with N-CoR. These experiments indicate that the interaction of huntingtin with N-CoR may have consequences for transcription of sets of genes under the control of N-CoR which may contribute to the pathology of HD.

Program Nr: 1550 from the 1999 ASHG Annual Meeting

Allelic Variants of Candidate Genes TGFA, TGFB3, and MSX1 and Orofacial Clefting in Norway: A Case-Parent Triad Approach. *A. JUGESSUR[1,2,3], R.T. LIE[2], A. WILCOX[4], F. ÅBYHOLM[5], H. VINDENES[5], J.C. MURRAY[3]*. 1) Center for Molecular Medicine, Haukeland Hospital, Bergen, Norway; 2) Division for Medical Statistics, Univ. of Bergen, Bergen, Norway; 3) Dept Pediatrics, Univ Iowa, Iowa City, IA; 4) Epidemiology Branch, NIEHS, Research Triangle Park, NC; 5) Norwegian Radium Hospital, Oslo, Norway.

Orofacial clefts are common congenital malformations occurring in approximately 2/1000 live births. Past studies have suggested multifactorial causation, with both genetic and environmental origins. The most studied allele associated with facial clefts has been transforming growth factor alpha, TGFA, although results are not consistent across studies. More recent reports have suggested additional associations of facial clefts with transforming growth factor beta 3, TGFB3, and MSX1 (a member of the family of Drosophila Msh, muscle segment homeobox). New candidate genes have been proposed, including genes for growth and signaling factors, and extracellular matrix proteins. The present study was based on DNA collected from case-parent triads, which eliminates possible bias from population admixture. A linkage disequilibrium-based analysis was used to detect non-Mendelian transmission of TGFA, TGFB3, RARA and MSX1 alleles in a total of 126 Norwegian nuclear triads. To date, we have completed the genotyping for TGFB3 and MSX1 using CA-repeat markers. The results indicate no formal statistical interaction between these two genes and either isolated cleft lip (NSCLO) or isolated cleft lip and palate (NSCL/P). However for MSX1, a similar pattern of allele representation was observed as in a previous study in Caucasian CL/P patients ($p = 0.07$). To investigate this further, another polymorphic variant of MSX1, MSX1-1.3, is currently being genotyped in our case-parent triads. Prospective analyses include screening a subset of the cases for variants in the RARA gene, and investigating environmental interactions such as smoking and folic acid metabolism in the etiology of clefts.

Program Nr: 1551 from the 1999 ASHG Annual Meeting

Agenesis of the corpus callosum associated with a chromosome (4;14) translocation. *D. Kamnasaran[1], M. Muenke[2], P.C.M. O'Brien[3], M.A. Ferguson-Smith[3], D.W. Cox[1].* 1) Medical Genetics, University of Alberta, Edmonton, Alberta, Canada; 2) Children's Hospital of Philadelphia and MBG, NHGRI, NIH, Bethesda, MD, U.S.A; 3) Clinical Veterinary Medicine,Centre for Veterinary Science,Cambridge, U.K.

Disorders such as holoprosencephaly and autosomal dominant and recessive deafness syndromes are associated with the chromosome 14q12-q13 cytogenetic region. We report a proband with a 46,XY,t(4;14)(q25;q13) karyotype who has agenesis of the corpus callosum, microcephaly, low set ears, feeding problems and failure to thrive. We have mapped the proband's translocation breakpoint between the markers AFM200ZH4 and D14S306 on chromosome 14 and between the markers D4S2449 and D4S3167 on chromosome 4 by PCR analysis of flow sorted chromosomes. We have assembled a 3 Mb YAC contig that spans the translocation breakpoint on chromosome 14 by selecting CEPH YAC clones that are positive for markers localized to the interval. This was done in order to map, by PCR, all known genes that have been binned to the 14q13 region and to analyze potential candidate genes. Six known genes, PAX9, TTF1, HNF3A, SEC23, SDK3 and RPS14 mapped onto the YAC contig. Of the genes mapped at the 14q13 translocation breakpoint, TTF1 is a potential candidate for the agenesis of the corpus callosum observed in the proband. We have also analyzed PITX2 at the chromosome 4q25 region as another potential candidate gene for agenesis of the corpus callosum. PITX2 is the gene for Rieger syndrome. A putative deletion within PITX2 was found by PCR analysis of flow sorted chromosomes with primer pairs that amplified different exons of the gene. PITX2 was physically estimated to map at least 1.2 Mb proximal to the translocation breakpoint on chromosome 4 by mapping on CEPH YAC clones binned to 4q25. The presence of two breaks at 4q25 implies a possible inversion at this region.

Program Nr: 1552 from the 1999 ASHG Annual Meeting

A putative *Drosophila* homolog of the *Huntington disease* gene. *C.A. Karlovich[1], Z. Li[1], M.P. Fish[2], M.P. Scott[2], R.M. Myers[1]*. 1) Dept. of Genetics, Stanford University, Stanford, CA; 2) Dept. of Genetics and Developmental Biology, Stanford University, Stanford, Stanford, CA.

The *Huntington disease (HD)* gene encodes a protein, huntingtin, with no known function and no detectable sequence similarity to other proteins in current databases. To gain insight into the normal biological role of huntingtin, we isolated and sequenced a cDNA encoding a protein that is a likely homolog of the HD gene product in *Drosophila melanogaster*. We also determined the complete sequence of 43,125 contiguous base pairs of genomic DNA that encompasses the *Drosophila HD* gene, allowing the intron-exon structure and 5' and 3' flanking regions to be delineated. The predicted *Drosophila* huntingtin protein has 3,583 amino acids, which is several hundred amino acids larger than any other previously-characterized member of the HD family. Analysis of the genomic and cDNA sequences indicates that *Drosophila HD* has 29 exons, compared to the 67 exons present in vertebrate *HD* genes, and that *Drosophila* huntingtin lacks the polyglutamine and polyproline stretches present in its mammalian counterparts.

We have generated antibodies against several peptide regions from Drosophila HD. Using these antibodies, we find that the Drosophila HD protein is expressed in a broad range of developmental stages and in the adult. The *Drosophila HD* mRNA is similarly expressed. Thus, the temporal pattern of expression of both protein and mRNA in the fly is similar to that observed for mammalian huntingtins.

In contrast to vertebrate huntingtins, which have a high degree of sequence similarity to one another, the Drosophila HD protein shows only low overall similarity to other members of the HD family. This low sequence conservation makes it possible to discern five regions of relatively high similarity from multiple sequence alignments between *Drosophila* and vertebrate huntingtins. These regions may define functionally important domains within the protein.

Program Nr: 1553 from the 1999 ASHG Annual Meeting

Trinucleotide repeat for SCA8 on 13q21: super expansion in psychosis individuals unaffected by ataxia. *J.L. Kennedy[1], M.L. Neves-Pereira[1], A.D. Paterson[1], E. Yamamoto[1], S.V. Parikh[1], F. Macciardi[1], A. Petronis[1], J.B Vincent[1,2].* 1) Neurogenetics Section, Clarke Inst, Toronto, ON, Canada; 2) Dept. Genetics, Hospital for Sick Children, Toronto, ON, Canada.

Increased frequency of large CAG/CTG trinucleotide repeat tracts in individuals affected with schizophrenia (SCZ) and bipolar affective disorder (BPAD) has previously been reported. Studies of two unstable CAG/CTG repeats (SEF1-2B and ERDA1) indicate that the majority of individuals with large repeats (detected by RED) have large repeat alleles at these loci, but that there is no association of large alleles with either BPAD or SCZ. Using RED, we have identified from amongst our sample a BPAD individual with a very large (>690bp) trinucleotide repeat tract at a third site. From this individual s DNA, we have cloned a new highly polymorphic trinucleotide repeat, TGC13-7a, comprised of [TAC]n [TGC]n, which isexpanded (~1800bp) in this patient. The repeat region localizes to chromosome 13q21, and has recently been shown to be the cause of spinocerebellar ataxia type 8, where alleles with greater than ~107 repeats are thought to be pathogenic. We observed no significant differences in allele distribution between 100 BPAD patients and 100 matched controls (p=0.06), and no transmission disequilibrium in 93 BPAD trios (p=0.49) or 54 SCZ trios (p=0.46). We have now identified a number of other individuals with very large expansions at TGC13-7a/SCA8: a SCZ individual with ~550 repeats, a SCZ individual with 115 repeats and unaffected father with ~700 repeats, none of whom are affected by spinocerebellar ataxia. Whilst these expansions are relatively rare (~0.5% of all individuals tested), it is clearly much more common than the frequency of all spinocerebellar ataxia cases (0.01%), let alone SCA8. Super expansions at TGC13-7a/SCA8 may be allelic variants that predispose psychosis via a different molecular mechanism to the more modest expansion alleles reported for SCA8, or may be completely unrelated to disease.

Program Nr: 1554 from the 1999 ASHG Annual Meeting

Using 2D gel analysis of wildtype and *Fmr1* knockout mouse hippocampus to identify differentially expressed proteins. *L.L. Kirkpatrick, D.L. Nelson.* Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX.

Fragile X syndrome results from mutations in the X-linked *FMR1* gene. The most common mutation is expansion and hypermethylation of a CGG repeat in the 5UTR of *FMR1* which blocks transcription and results in the loss of FMR1 protein (FMRP). FMRP has two KH domains and an RGG box, protein motifs thought to mediate RNA binding, and studies have shown that FMRP binds RNA in vitro. This suggests that FMRP may be involved in some aspect of RNA metabolism, perhaps in targeting, transport, or translation regulation. A necessary first step towards understanding how the absence of FMRP leads to mental retardation is the identification of mRNAs and/or proteins with which FMRP interacts. We have used 2D gel analysis in an effort to identify proteins whose expression levels are changed in the absence of FMRP. Wildtype and *Fmr1* knockout mouse hippocampus samples were subjected to 2D SDS-PAGE and differences in protein spots were then identified using a computerized comparison. The spots were cut from the gels and subjected to mass spectroscopy fingerprinting for identification. Using this method, we have identified NFL and TOAD64 as being increased in knockout hippocampus, and HSC70 as increased in wildtype hippocampus. Northern blot, western blot, and immunocytochemical analyses are being used to validate the 2D gel results.

Program Nr: 1555 from the 1999 ASHG Annual Meeting

CAG/CTG repeats in auotimmune disease: a possible role in rheumatoid arthritis? *W. Klitz[1], L. Barcellos[2], Q-p. Yuan[3], K. Lindblad[3], M. Schalling[3], L. Field[4], M. Sheldin[5]*. 1) Sch Public Health, Univ California, Berkeley, CA; 2) Dept Neurology, Univ California, San Francisco, CA; 3) Cntr Molecular Medicine, Karolinska Hosp, Stockholm, Sweden; 4) Dept Medical Genetics, Univ Calgary, Calgary, Canada; 5) Rowe Program in Genetics, Univ California, Davis, CA.

Spontaneous expansions of trinucleotide repeats have now been shown to be responsible for at least a dozen diseases, many of them involving genes affecting the nervous system. In many of these disorders, anticipation, an increased severity and earlier age of onset from one generation to the next, is seen. Because the discovery of non-HLA genetic components of autoimmune disease has been frustratingly slow, a non-calssical genetic mechanism seemed worth pursuing as an underlying biological contributor to autoimmune disease. We examined individuals scored for CAG/CTG exapnded repeat size (³120nt) sampled from three autoimmune diseases--insulin dependent diabetes mellitus (IDDM), multiple sclerosis (MS), and rheumatoid arthritis (RA)--and compared them to a control sample.

Repeat sizes of two known locations of CAG/CTG expansions (ERDA1 and CTG18.1) were phenotyped by PCR and all expansions phenotyped by the repeat expansion detection method. None of the the three diseases differed in phenotype frequencies of expanded repeats compared to the controls. Age of onset and HLA DR typing information was also examined for each disease. The IDDM and MS samples showed no effects with these two variables, but for the RA sample earlier age of onset ($p = 0.019$) and absence of the predisposing shared DR epitope ($p = 0.036$) were each associated with increased frequency of CAG/CTG expansions. These observations suggest that trinucleotide repeat expansions may play a role in rheumatoid arthritis, and await confirmation in an independent RA patient sample.

Program Nr: 1556 from the 1999 ASHG Annual Meeting

Tissue specificity in the mitochondrial tRNA processing implicates the clinical heterogeneity in mitochondrial disorders. *Y. Koga, Y. Akita, R. Iwanaga, H. Kato.* Dept Pediatrics & Child Health, Kurume Univ Sch Medicine, Kurume, Fukuoka, Japan.

In order to know the pathological meaning of tissue specific processing at the mitochondrial tRNALeu(UUR) boundary in mitochondrial myopathy, we analyzed the percentage of processing intermediates. Total RNAs were isolated from various human tissues from 8 normal individuals, and from 5 patients. Three patients have been diagnosed MELAS having A3243G mutation, one patient diagnosed Leigh disease having A3243G mutation, and one patient diagnosed mitochondrial encephalomyopathy and cardiomyopathy having T3303C mutation in the mitochondrial tRNALeu(UUR) gene. We analyzed the percentage of point mutation in RNA 19 fraction in RNAs from muscles, using rTth reverse transcriptase PCR-RFLP methods. We also analyzed the 5'- or 3'-end of tRNALeu(UUR) by primer-extension or S1-digestion method. The RNA 19 is recognized in normal human tissues including muscle, heart, brain, kidney, liver, spleen and uterus, ranging 3.5%, 11.6%, 13.6%, 8.4%, 4.2%, 1.7% and 6.5% of their total ND 1 signal, respectively. 3'-end of tRNALeu(UUR) portion is processed much earlier than 5'-end in most of the human tissues, while in the fibroblasts, 5'-end of tRNALeu(UUR) portion is processed much earlier than 3'-end. In patients, we observed significantly increased levels of RNA 19 in muscles from all patients examined ; 3 MELAS (5 - 8 times more than that of the control), Leigh (8 times) and T3303C (, which is well harmonized with the percentage of mutation analyzed in their mitochondrial DNA from muscles. The proportion of mutated RNA in RNA19 fraction always higher than that in DNA fraction, suggesting that these point mutations located in the tRNALeu(UUR) gene exhibited dominant negative effects on the mitochondrial RNA processing events, resulting in the accumulation of RNA 19 transcripts in these patients. This accumulation of RNA 19 seems to be specific for the tRNALeu(UUR) mutation and related to the pathogenic mechanism of MELAS.

Program Nr: 1557 from the 1999 ASHG Annual Meeting

Sequence-specific and non-specific nucleotide binding activities of the ORF1 protein from mouse LINE-1. *V.O. Kolosha, S.L. Martin.* Dept. of Cellular and Structural Biology, University of Colorado School of Medicine, Denver, CO.

LINE elements and other non-LTR retrotransposons are widely dispersed in nature. In humans, transposition of LINE elements in germ cells or early embryos can disrupt essential genes, leading to heritable disease. Mammalian LINE-1 encodes two proteins that are required for transposition. The ORF1 gene product is a nucleic acid binding protein. It appears to "coat" the L1 RNA forming a ribonucleoprotein particle, although its exact role in transposition remains to be elucidated. Contradictory data establishes the human protein as a sequence-specific RNA binding protein, whereas the mouse homolog is a sequence non-specific, single-stranded nucleic acid binding protein. In this study, the mouse L1 ORF1 protein is purified in soluable form from both insect cells and *E. coli* to apparent homogeneity. The results obtained from several independent assays confirm our earlier observations that the ORF1 protein binds single-stranded nucleic acids non-specifically. In addition, these studies extend those observations to demonstrate the presence of a single, relatively high-affinity site for a specific sequence within the mouse L1 RNA. Both baculo- and bacterially-expressed ORF1 proteins coimmunoprecipitate a specific 38 nucleotide fragment from several, overlapping RNAase T1-digested, L1 *in vitro* transcripts. This analysis allows the unambiguous identification of the bound fragment. The sequence is U-rich (U-content 42% in L1Md-A2), and lies just 3' of the AUG codon at the beginning of the ORF2 coding sequence, homologous to one of two high-affinity sites identified for the human ORF1 protein in human L1 RNA. The fact that the structure of this short region of the ~7kb L1 RNA is sufficient to be recognized by the ORF1 protein is supported by co-immunoprecipitation of a 43 nucleotide transcript containing this region. The high-affinity interaction of ORF1 protein with the L1 RNA may nucleate the coating of L1 RNA by ORF1 protein. Once the first molecule of ORF1p is bound, the lower affinity, non-specific interactions and protein-protein interactions would allow the RNA to be covered by ORF1p, forming the ribonucleoprotein particle.

Program Nr: 1558 from the 1999 ASHG Annual Meeting

Differential display analysis of inducible N2a cells stably expressing huntingtin exon 1 with normal and expanded polyglutamine region . *S.E. Kotliarova, G.H. Wang, Y. Kamide, M.A. Zemskova, N. Nukina.* Lab.for CAG repeat diseases, Brain Science Institute, RIKEN, Wako-shi, Saitama, Japan.

Huntington disease is caused by expansion of CAG repeats in huntingtin exon 1 that results in abnormal protein with expanded polyglutamine (pQ) tract. This triggers the chain of events resulting in formation of nuclear inclusions and neuronal cell death.

To study the factors, which may be involved in mechanism of Huntington disease, we have established the new model system of this disease inducible stable cell lines of mouse neuroblastoma HD16Q-23 (16pQ) and HD150Q-28 (150 pQ). The constructs containing fusion protein of Huntingtin exon 1 and GFP were used to generate these cell lines.

mRNA isolated from these cell lines was analysed by Differential Display (DD) technique . Several differentially expressed bands were detected in mRNA extracted on the third day after differentiation and induction, when the majority of HD150Q-28 cells were dying. Among differentially expressed cDNA several apoptosis-related genes were identified. The difference in expression for these genes was confirmed by semi-quantitive RT-PCR and TaqMan assay in cells harvested on the third day.

In addition we compared the pattern of expression level change for several genes during time course from 0 to 3 days in 0.5 day increments in cells expressing normal and expanded pQ .

Our data suggest that genes participating in programmed cell death are probably involved in the mechanism of cell death triggered by expanded pQ of huntingtin.

Program Nr: 1559 from the 1999 ASHG Annual Meeting

Linkage analysis of 3 novel *CD4* promoter polymorphisms in Danish IDDM multiplex families. *O.P. Kristiansen, F. Pociot, J. Johannesen, A.E. Karlsen, J. Nerup, T. Mandrup-Poulsen, DSGD, DIEGG.* Steno Diabetes Center, Gentofte, Denmark.

Recently, evidence for linkage between IDDM and the A4 (113bp) allele of a CTTTT-repeat in the *CD4* promoter was reported. The **aims** of the present study were to screen the *CD4* promoter for novel polymorphisms and to investigate for linkage between novel *CD4* polymorphisms and IDDM in the Danish population. **Methods and material**: SSCP screening of the *CD4* promoter (position 386®1523bp; M86525) was performed in 20 IDDM and 10 healthy subjects. The allelic variants in the SSCP polymorphic sites were identified by cycle sequencing. Linkage was investigated by the transmission disequilibrium test (TDT) in 146 sibpair and 103 parent-offspring IDDM families. Transmission to 418 affected and 249 non-affected offspring was observed. **Polymorphism screening**: Three novel polymorphisms were found: 1) a T®C substitution in pos. 474 bp, 2) a G®C substitution in pos. 1003 bp, and 3) a C®G substitution in pos. 1343bp. PCR based ([1]*BslI* RFLP, [2]*MspI* RFLP and [3]a mutagenically separated (MS)) assays for the polymorphisms were established. **Linkage analysis**: The G-allele of the *BslI* (pos. 474 bp) polymorphism was in complete linkage disequilibrium with allele A8 (88bp) of the *CD4* (CTTTT)-repeat, known not to be linked to IDDM. Both the *MspI* (pos. 1003 bp) and the *BsaXI* (pos. 1343 bp) alleles showed random transmission to the IDDM offspring, but a non-significant tendency to skewed transmission was observed for both polymorphisms: 80 (55%) G- and 66 (45%) C-alleles (P_{tdt}=0.25) and 160 (54%) C- and 134 (46%) G-alleles (P_{tdt}=0.13) were transmitted, respectively. A *MspI-BsaXI* haplotype was established. Transmission of the 3 identified haplotypes G-C, C-G and G-G were 55% (164/300; P_{tdt}=0.11), 43% (65/151; P_{tdt}=0.09) and 48% (113/233; P_{tdt}=0.6). In **conclusion**, we have identified 3 novel polymorphisms in the *CD4* promoter. None of the found polymorphisms or the resulting *MspI-BsaXI* haplotype were significantly linked to IDDM in the Danish population. Thus, it is less likely that any of the novel polymorphisms confer susceptibility to IDDM, although the region is linked (*CD4* (CTTTT)-repeat) to IDDM.

Program Nr: 1560 from the 1999 ASHG Annual Meeting

A novel 4.4kb mtDNA deletion underscores clinical heterogeneity in mitochondrial DNA deletion disorders. *F.L. Lacbawan[1], C.J. Tifft[2], M. Amaya[2], M. Pennybacker[3], S. Weinstein[4], L.C. Wong[3].* 1) NHGRI,NIH, Bethesda, MD; 2) Dept of Medical Genetics, Children's National Medical Center, Washington DC; 3) Institute for Molecular and Human Genetics, Georgetown University, Washington DC; 4) Dept of Neurology,Children's National Medical Center, Washington DC.

Among the mitochondrial DNA deletion (mtDNA del) disorders, chronic progressive external ophthalmophlegia (CPEO), Kearne-Sayre syndrome (KSS), Pearson marrow-pancreas syndrome, diabetes insipidus, diabetes mellitus, optic atrophy, and deafness(DIDMOAD)and cyclic vomiting syndrome are relatively well-defined clinically. However with more reported cases, it is evident that a whole spectrum of disease severity can be associated with similar deletions of mtDNA and there is no correlation of phenotype to the size of mtDNA deletion. We report an 8 yo Hispanic girl with short stature, ataxia, dysphagia, and aphasia who had transfusion-dependent macrocytic anemia during infancy and early childhood and recurrent bacterial and fungal infections. Extensive hematologic and immunologic work-up were unrevealing. Therapeutic trials of folate, folinic, and vitamin B12 were unsuccessful however her anemia apparently resolved after 2 years. Later, gait problems, exercise intolerance and chronic episodic vomiting were also noted. A lactate/pyruvate ratio was normal but MRI-spectroscopy revealed a large lactate peak. A cranial computed tomography scan showed bilateral basal ganglia calcifications and extensive frontal lobe white matter changes. Ragged red fibers were present on muscle biopsy. A 4.4 kb deletion was identified by Southern blot analysis of muscle mtDNA. Subsequent PCR and sequencing analyses localized the deletion to be between nucleotides 10559 and 14981. The same heteroplasmic deletion was demonstrated in blood, buccal cells and hair follicles. Although large deletions are usually sporadic, analysis of maternal DNA is in progress. The constellation of clinical features in this patient does not point to a single mtDNA del syndrome. This case further illustrates the clinical heterogeneity with mitochondrial disorders, and encourages physicians to maintain a high index of suspicion in patients with atypical findings.

Program Nr: 1561 from the 1999 ASHG Annual Meeting

Is the myotonic dystrophy protein kinase (DMPK) a muscle-specific protein? *L.T. Lam, Y.N.C. Pham*, N.T. Man, G.E. Morris.* MRIC Biochemistry Group, North East Wales Institute, WREXHAM, UK.

Myotonic dystrophy (DM) is a multi-systemic disorder caused by a triplet repeat expansion which affects three different genes, DMPK, Six5 (or DMAHP) and gene 59. The cell and tissue distribution of their protein products may help us to understand their different roles in DM pathogenesis. Unfortunately, many antibodies raised against DMPK are subject to cross-reactions which have lead to confusion over its size (72-80kD or 52-55kD) and localisation.
In an earlier study (Pham et al, Hum. Mol. Genet. 7 (1999) 1957), a new panel of ten monoclonal antibodies (mAbs) was used to show that a 72-80kD protein is the main product of the human DMPK gene. Only one mAb, MANDM1, recognised rabbit or mouse DMPK and this was used to show a 72-80kD protein in all rabbit tissues tested, including brain and lung. Using human tissues, however, we now show that most mAbs against DMPK do not react with any protein on Western blots of brain, lung or skin fibroblasts, though they all recognise the same DMPK band in skeletal and cardiac muscle. This suggests that DMPK is expressed at high levels only in skeletal and cardiac muscle, a result which is consistent with in situ hybridisation studies of DMPK mRNA (Jansen et al, Nat. Genet. 13 (1996) 316). The misleading results obtained with mAb MANDM1 may result from cross-reaction with a protein of similar size, possibly a related protein kinase.
A subset of 6 mAbs from the original panel has now been identified which recognise a DMPK band only in skeletal and cardiac muscle and these mAbs recognise different epitopes in both the catalytic and coil domains.
Supported by the Muscular Dystrophy Campaign..

Program Nr: 1562 from the 1999 ASHG Annual Meeting

Haplotype and AGG-interspersion analysis of *FMR1* $(CGG)_n$ alleles in the Danish population: implications for multiple mutational pathways towards fragile X alleles. *L.A. Larsen[1], J.S.M. Armstrong[2], K. Grønskov[2], H. Hjalgrim[2], J. Macpherson[3], K. Brøndum-Nielsen[2], L. Hasholt[4], B. Nørgaard-Pedersen[1], J. Vuust[1]*. 1) Department of Clinical Biochemistry, Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen, Denmark; 2) Department of Medical Genetics, The Department of Medical Genetics, The John F. Kennedy Institute, 7. Gl. Landevej, DK-2600 Glostrup, Denmark; 3) Wessex Regional Genetics Laboratory, Salisbury District Hospital, Salisbury, Wiltshire SP2 8BJ, United Kingdom; 4) Institute of Medical Biochemistry and Genetics, University of Copenhagen, The Panum Institute, Blegdamsvej 3, DK-2200 Copenhagen, Denmark.

The AGG interspersion pattern and flanking microsatellite markers and their association with instability of the *FMR1* $(CGG)_n$ repeat, involved in the fragile X syndrome, were analyzed in DNA from filter-paper blood spots randomly collected from the Danish newborn population. Comparison of DXS548-FRAXAC1 haplotype frequencies in the normal population and among fragile X patients suggested strong linkage disequilibrium between normal alleles and haplotype 7-3 and between fragile X alleles and haplotype 2-1 and 6-4. Comparison of the AGG interspersion pattern in 143 $(CGG)_n$ alleles, ranging from 34-62 CGG in size, and their associated haplotypes, indicates the existence of at least three mutational pathways from normal alleles towards fragile X alleles in the Danish population. Two subgroups of normal alleles, with internal sequences of $(CGG)_9AGG(CGG)_{20}$ and $(CGG)_9AGG(CGG)_{12}AGG(CGG)_9$, possibly predisposed for expansion, were identified in the data set. When alleles larger than 34 CGG were investigated, comparing the lengths of 3 uninterrupted CGG triplets (uCGG), we found that alleles associated with haplotype 2-1 and 6-4 contains significantly longer stretches of uCGG than alleles associated with haplotype 7-3. Thus the data support that $(CGG)_n$ instability is correlated to the length of uCGG.

Program Nr: 1563 from the 1999 ASHG Annual Meeting

Effect of mutations on cytochrome P4501B1 (CYP1B1), the protein responsible for Primary Congenital Glaucoma. *F. Abou-Zahr, B.A. Bejjani, F.A.E. Kruyt, R.A. Lewis, J.R. Lupski, H. Youssoufian.* Molecular & Human Genetics, Baylor College of Medicine, Houston, TX.

CYP1B1 is a dioxin-inducible member of the cytochrome P450 gene superfamily with an as yet undefined role in ocular development. CYP1B1 and other drug-metabolizing enzymes seem to regulate the steady state of biologically active molecules (e.g. hormones) that affect growth and differentiation. Previous studies have shown that mutations in *CYP1B1* cause primary congenital glaucoma. We developed cell culture and in vitro assays to evaluate the effect of patient-derived mutations on the expression, localization and function of CYP1B1. In vitro-generated mutants (5 missense mutations and one 10-bp deletion) were cloned in the mammalian expression vector pcDNA3. Similar to wild-type CYP1B1, the missense mutations encode full-length proteins that are localized to microsomes in the cytoplasm. By contrast, the 10 bp deletion produced an undetectable protein. Cell lysates were prepared and incubated with varying amounts of Estradiol (E2), a known substrate for CYP1B1 enzymatic action. Conversion of E2 to 4 OH-E2 is being analyzed by GC/MS. Interactions of CYP1B1 with other enzymes involved in xenobiotic metabolism are currently being investigated. An understanding of the mechanism of CYP1B1 function provides insights into the effects of endogenous morphogens or xenobiotics on ocular development.

Program Nr: 1564 from the 1999 ASHG Annual Meeting

A novel mutation in ATP7A deletes the di-leucine targeting motif in a family with occipital horn syndrome. *A.N. Adam, S.L. Dagenais, J.W. Innis, T.W. Glover.* Pediatrics and Human Genetics, University of Michigan, Ann Arbor, MI.

Occipital horn syndrome (OHS) is an X-linked recessive disorder of copper metabolism allelic to the more severe Menkes syndrome. Both syndromes result from mutations in a P-type ATPase known as ATP7A. Affected OHS males are deficient in the cuproenzyme lysyl oxidase, an enzyme which initiates cross-linking of collagen and elastin. The resulting phenotype involves primarily connective tissue, and leads to characteristics such as hyperelastic skin, hypermobile digital joints, bony abnormalities including protuberances from the occiput, bladder diverticulae and inguinal herniae. Based on radiographic examinations, serum copper levels and phenotypic manifestations, we have identified a family in which two brothers and their maternal uncle are affected with OHS. RT-PCR analysis did not reveal aberrant splicing, and Northern analysis of fibroblast mRNA did not show differences in ATP7A levels in affected individuals. Sequence analysis at the genomic level revealed a single base deletion in exon 23 of the two affected siblings and their carrier mother, while the unaffected brother and a control subject had no deletion. The deletion causes a frameshift which is of interest for several reasons. Conceptual translation of the mutant message produces a truncated form of the ATP7A protein which is missing the di-leucine L1487L1488, recently reported to be a motif necessary for steady state localization of ATP7A to the *trans*-Golgi network (TGN). Loss of this motif in artificial ATP7A constructs results in the accumulation of ATP7A on the plasma membrane due to loss of the proteins ability to recycle back to the Golgi. Of interest is the fact that this loss produces the relatively mild phenotype of OHS rather than a more severe phenotype. Previously we localized ATP7A to the TGN using antibodies generated against the first four metal binding domains of ATP7A. Similar examination of cells from OHS family members should reveal whether this mutation causes a shift in steady state localization of ATP7A from the TGN to the plasma membrane. These studies will further our understanding of di-leucine targeting motifs and how their function is manifested phenotypically.

Program Nr: 1565 from the 1999 ASHG Annual Meeting

Novel mutations in the duplicated region of the Polycystic Kidney Disease 1 (PKD1) gene reveals supporting evidence for the gene conversion theory in this gene. *A.R. Afzal[1], R.N. Florêncio[2], A. Saggar-Malik[1], R. Taylor[1], S. Jeffery[1]*. 1) Dept. of Medical Genetics, St. Georges Hospital Medical School, London, United Kingdom; 2) Ribeirao Preto Medical School, University of São Paulo, Brazil.

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is one of the most common single-gene disorders of humans, and is the most common frequent inherited form of cystic kidney disease. It is estimated that ~85% of ADPKD is due to mutations in the PKD1 gene, that is located on chromosome 16p13.3. Mutation analysis in the PKD1 gene is difficult because more than two thirds of the gene is reiterated several times on chromosome 16p13.1. In this study, mutation screening in 90 ADPKD1 patients was carried out on exons in the duplicated region of the PKD1 gene (23-34) using genomic long range PCR followed by nested PCR and SSCP, and finally cycle sequencing. Two novel non-conservative missense mutations were detected in exons 25 and 31. Moreover, 4 conservative mutations were found in exons 24, 25, 29, and 33, that in exon 25 having been reported previously. Furthermore, one splicing mutation which is expected to cause skipping of exon 30 was detected in one case. In 14 cases polymorphisms G>C9406 and T>C9407 reported by Watnick et al., 1997, were also found. Comparison between some of these changes and published sequences from the homologous genes on 16p13.1 revealed supporting evidence for the gene conversion theory as a mechanism responsible for mutation in PKD1.

Program Nr: 1566 from the 1999 ASHG Annual Meeting

Genetic heterogeneity in autosomal dominant recurrent fevers. *E. Aganna[1], M.F. McDermott[1], D. Booth[2], B.W. Ogunkolade[1], G.A. Hitman[1], P.N. Hawkins[2]*. 1) Medical Unit, Royal London Hospital, London, UK; 2) Immunological Medicine, Hammersmith Hospital, London, UK.

TNFR1 associated periodic syndromes (TRAPS) have been described in a number of different ethnic groups, the majority of which are Northern European in origin. The TRAPS phenotype, inherited as an autosomal dominant trait, is characterised by recurrent attacks of fever associated with variable manifestations of abdominal pain, and synovial and cutaneous inflammation. A multiplex Indian family that includes at least 8 living family members suffering from intermittent episodes of fevers lasting >1 week, limb pains, rashes, peri-orbital oedema and serositis was studied. In addition 4 members of this family have documented systemic AA amyloidosis. As this phenotype strongly resembles TRAPS we used an informative microsatellite marker from the first intron of TNFR1 in addition to D12S99 and D12S77 which flank this locus to genotype all available members, both affected and unaffected. Two-point and multipoint lod scores definitively excluded linkage to TNFR1 and a haplotype study confirmed these findings. Sequencing of exons 1-9 and flanking sequences of TNFR1 revealed no mutations. To further exclude the possibility of pseudo-dominant familial Mediterranean fever (FMF) we also used markers flanking the MEFV locus on chromosome 16p13.3, and linkage was also definitively excluded. Thus there is evidence of genetic heterogeneity within the spectrum of autosomal dominant recurrent fevers as disease in this family is not linked to either TNFR1 or MEFV.

Program Nr: 1567 from the 1999 ASHG Annual Meeting

Functional analysis and identification of the DNA binding site of the *Hairless* gene product. *V.M. Aita[1], H. Uyttendaele[2], P.B. Cserhalmi-Friedman[2], T.C. Gilliam[1,3], A.M. Christiano[1,2]*. 1) Department of Genetics & Development, Columbia University, New York, NY; 2) Department of Dermatology, Columbia University, New York, NY; 3) Columbia Genome Center, Columbia University, New York, NY.

Investigations into the relationship between the structure and function of the *hairless* (*hr*) gene provide valuable insight into the specific nature of the transcriptional regulation mediated by this gene. *Hr* is an essential regulator of the hair cycle and has been implicated in a form of congenital atrichia in both humans (OMIM#209500) and hairless and rhino mice. In humans, distinct mutations in the *hr* gene have been observed in families from diverse ethnic origins including Pakistani, Irish, Arab Palestinian, Japanese and Polish. Of particular interest, the mutation in the Polish family is a missense mutation changing the invariant third cysteine residue of the zinc-finger binding domain to a glycine. In rhino mice, we have identified four distinct nonsense mutations leading to an absense of functional protein, which results in a dysregulation of apoptosis at the first catagen phase of the adult hair cycle and subsequent destruction of the hair follicle. *Hr* has been identified as a putative transcription factor with structural homology to the GATA family of transcription factors since it contains a single zinc-finger domain with high homology to the type found in GATA transcription factor family members. We demonstrate *Hr* binds DNA by western blot analysis of proteins captured via their binding to DNA-cepharose beads incubated with total protein lysates from 293T cells overexpressing the *hr* gene. Binding site selection assays such as the CASTing method will elucidate the specific DNA binding sequence bound by *Hr* protein, which will be confirmed by gel shift assay. The recapitulation of the Polish family zinc-finger mutation by site-directed mutagenesis will be used to investigate a putative loss of DNA binding activity as the molecular basis for congenital atrichia in this family and facilitate a better understanding of *hr* gene function and genotype-phenotype correlations in humans and mice with this disorder.

Program Nr: 1568 from the 1999 ASHG Annual Meeting

Uroporphyrinogen III Synthase: Identification/Characterization of An Erythroid-Specific Promoter and Mutations Therein Causing Congenital Erythropoietic Porphyria. *G.I. Aizencang, C. Solis-Villa, L. Cunha, D.F. Bishop, K.H. Astrin, R.J. Desnick.* Department of Human Genetics, Mount Sinai School Medicine, New York, NY.

Mutations in the gene encoding the heme biosynthetic enzyme uroporphyrinogen III synthase (UROS) cause congenital erythropoietic porphyria (CEP). This autosomal recessive porphyria has a variable phenotype ranging from non-immune hydrops fetalis to a mild adult-onset form with only cutaneous lessions. To date, a common severe (C73R) and 17 other (mostly private) mutations causing CEP have been reported; however, ~15% of mutant alleles remain undetected. Thus, the human UROS genomic structure was determined revealing alternative promoters and two alternative 5'untranslated and nine coding exons which generated housekeeping and erythroid-specific transcripts. Sequencing of the erythroid-specific promoter of CEP patients with undefined alleles revealed three mutations in four patients: 1) a -70 T to C transversion altering a GATA-1 consensus sequence, 2) a -86 C to A transversion and 3) a -90 C to A transversion, the latter two mutations in a putative erythroid regulatory element. Luciferase reporter constructs with the -70C, -86A and -90A mutations transfected in K562 cells expressed 8.5, 49 and 13% of the normal promoter construct, respectively. Genotype/phenotype correlations were consistent with the *in vitro*expression of the patients' other alleles in *E. coli:* a fetus prenatally diagnosed with the -70C/C73R genotype had severe transfusion-dependent disease and was terminated; two unrelated adult women with the mild -86A lesion had moderate or only mild cutaneous disease, consistent with the severity of their other alleles, C73R or IVS2 $^{+1}$, respectively; and a male with the -90A/G225S genotype had moderately severe disease. Thus, discovery of an erythroid-specific promoter and lesions in the GATA-1 element and a novel regulatory element provide further understanding of the regulation of heme biosynthesis and the molecular pathology of CEP. The first two authors contributed equally to this work.

Program Nr: 1569 from the 1999 ASHG Annual Meeting

Multiple synostosis type 2 (SYNS2) maps to 20q11.2 and caused by a missense mutation in the growth/ differentiation factor 5 (GDF5). *A.N. Akarsu[1], T. Rezaie[2], M. Demirtas[1], D.D Farhud[3], M. Sarfarazi[4]*. 1) (TUBITAK) DNA/Cell Bank & Gene Research Lab, Child Hlth Inst/Hacettepe Univ, Ankara, Turkey; 2) Mehryad Medical Clinic, Tehran, Iran; 3) Department of Human Genetics, School of Public Health, Tehran University, Tehran, Iran; 4) Surgical Research Center, Department of Surgery, University of Connecticut Health Center, Farmington,CT.

GDF5 and Noggin are two important factors for proper skeletal development and joint formation. Mutations in these genes result in disruption of proper cartilage condensation and joint formation in several disorders such as chondrodysplasias and brachydactyly type C (GDF5) as well as symphalangism and multiple synostosis syndrome (Noggin). Herein, we report identification, ascertainment, mapping and mutation screening of a large autosomal dominant Iranian family (150 subjects; 61 affected) with clinical manifestation of tarsal-carpal coalition, humero-radial synostosis, brachydactyly and proximal symphalangism. These findings are mainly consistent with the Pearlman syndrome, but considerable overlaps also persist with other types of multiple synostosis syndromes. By using 38 informative meioses (21-phase known), the Noggin gene on 17q22 and many other candidate genes were first excluded. Eventually, significant linkage was obtained with DNA markers on 20q11.2. The highest LOD score was observed with D20S200 (Z=13.58 at theta=0.0), followed by D20S106 (Z=12.37) and D20S890 (Z=10.46). Affected crossovers positioned the disease gene between D20S844 and D20S478, a region that contained the GDF5 gene. Mutation screening of the proband identified a heterozygous missense mutation in a highly conserved region of GDF5 (S475N) that is co-segregated with the SYNS2 phenotype in 39 affected and 27 normal members. This mutation has not been detected in 182 unrelated control chromosomes. In conclusion, our result maps a new locus for SYNS2, identifies mutation in the GDF5 gene and further rejects a previous assumption that various types of SYNSs are due to the same genetic defect. Furthermore, this provides an opportunity for re-classification of these phenotypes using the existing molecular evidence.

Program Nr: 1570 from the 1999 ASHG Annual Meeting

TNF receptor associated periodic fever syndromes (TRAPS): novel TNFR1 mutations and early experience with etanercept therapy. *I. Aksentijevich[1], J. Galon[1], MF. McDermott[2], R. Ortman[1], JJ. O'Shea[1], DL. Kastner[1].* 1) ARB/NIAMS, NIH, Bethesda, MD; 2) Royal London Hospital, UK.

Mutations in the extracellular domains of the 55 kDa TNF receptor (TNFR1) define a family of autoinflammatory syndromes (TRAPS, TNF receptor-associated periodic syndromes) that are characterized by prolonged (>1 wk) periodic fevers, sterile peritonitis, pleurisy, arthritis, skin rash, and/or conjunctivitis; some patients also develop systemic AA amyloidosis. We have previously described 6 disease-associated TNFR1 mutations, 5 of which disrupt extracellular cysteines involved in disulfide bonds (Cell 97:133). For patients with the C52F mutation, there is diminished TNFR1 cleavage following cellular activation, with a commensurate reduction in levels of potentially antagonistic soluble receptor, thereby possibly explaining the autoinflammatory phenotype. In a survey of additional patients with periodic fevers, we have identified 4 new TNFR1 mutations: c.193-14G > A, H22T, C30S, and C33G. The first mutation has been found in two families of different ethnic background and the latter 2 mutations occur at residues at which we had already identified other missense mutations (C30R and C33Y). Another family had a T50M mutation, which we had previously seen in 2 other families. Of the 10 known mutations, c.193-14G>A and T50M are the only ones we have seen in more than 1 family. Functional analyses of leukocytes from two C33G patients indicated the same defect in TNFR1 shedding that had been observed in the C52F family. In 3 families and 4 sporadic cases with compatible clinical presentations, we have not yet identified TNFR1 mutations, despite genomic sequencing of the exons encoding the extracellular domains. Based on the proposed mechanism of disease, we have begun treatment of 3 members of the family bearing the C52F mutation with soluble p75 TNF receptor: Fc fusion protein (etanercept). In the one patient for whom followup clinical and laboratory data are available, there has been marked improvement, both in subjective measures and in a panel of acute phase reactants.

Program Nr: 1571 from the 1999 ASHG Annual Meeting

Construction of a sequence-ready 1.5 Mb PAC contig covering the whole OPA1 candidate region on chromosome 3q28-q29. *C. Alexander[1], G. Auburger[2]*. 1) Neurogenetics Lab, Dept. Neurology, University Hospital, Duesseldorf, Germany; 2) NIH, Bethesda, MD.

Autosomal dominant optic atrophy (Kjers type) is the most common form of all inherited optic atrophies with a prevalence of 1: 12.000 in certain regions. Patients suffer from a variable degree of visual loss ranging from subjectively unnoticed to legally blind. Histopathological findings point to a degeneration of retinal ganglion cells. Linkage analysis in various families affected by Kjers type optic atrophy has defined the microsatellites D3S3669 and D3S3562 as flanking markers for the disease critical region on chromosome 3q28-q29 of 1-2 cM. Building upon a previously constructed YAC contig we have established a 1.5 Mb sequence-ready PAC contig. It was constructed by an initial hybridisation screening of a high-density spotted genomic PAC library using STSs and probes derived from the YAC contig and subsequent filling of gaps by chromosomal walking. The clone order was defined by STS-PAC-Mapping and confirmed by Alu-fingerprint-PCR. The minimal tiling path covering the whole OPA1 candidate region comprises 11 PACs and the minimum depth is 3 PACs. The ends of all 120 PACs contained in the contig were sequenced and analysed by the BLAST software package. 75 new PCR-able STSs were developed leading to the very high STS-density of 1 STS every 18 kb on average throughout the whole contig. 7 ESTs/THCs were finemapped into the disease critical interval. We believe that the construction of this PAC contig is an important step in facilitating the cloning of the OPA1 gene and in the identification of further genes within this genetic interval.

Program Nr: 1572 from the 1999 ASHG Annual Meeting

Tuberous sclerosis 1 (*TSC1*) gene mutation screening by Denaturing High-Performance Liquid Chromatography (DHPLC). *A. Allavena, S. Padovan, L. Longa, S. Polidoro, A. Brusco, N. Migone.* Dept. of Genetics, Biology and Biochemistry, Univ. Torino; CNR-CIOS, Torino, Italy.

Mutation screening of the *TSC1* and *TSC2* genes responsible for tuberous sclerosis is a time-consuming challenge (overall, 62 exons) that has been so far accomplished by sequential, multi-method strategies, with a combined detection rate generally below 75%, even in presence of positive linkage to either locus. Recently, looking for an automated method we shifted to DHPLC, a technique which should be able to separate homo and heteroduplex DNA on alkilated non-porous particles under partial denaturing conditions. First, 23 samples known to carry *TSC1* or *TSC2* mutations (8 single nt substitutions, 1 nt insertion, 7 micro deletions) or polymorphisms (7) as previously shown by PTT, heteroduplex-polyacrylamide analysis (HD) or fluorescence assisted mismatch analysis (FAMA), were all confirmed by DHPLC, suggesting a sensitivity threshold equal or superior to standard methods. At present, we have set up the conditions for *TSC1* (23 amplicons, 7 of which requiring two different temperatures), and screened 41 patients who a) did not show major rearrangement in either gene at southern, and b) were *TSC2*-negative at PTT, and *TSC1*-negative at HD. This sample included 2 *TSC1*-assigned patients: 1 by linkage and 1 by LOH found in a giant astrocytoma. Briefly, two novel, de novo mutations and one polymorphism were found in *TSC1*. The rarity of *TSC1* involvement in this sample is not unexpected since the prevalence of *TSC1* mutations in unselected, i.e. mostly sporadic, patients is about 10%. Interestingly, one of the two mutations is a missense (L72P) in a conserved position in drosophila and rat. All 93 *TSC1* defects known so far are frameshifs mutations. The full screening of *TSC2* and the re-analysis of a few *TSC1* exons at different temperatures are under way in this patient in order to exclude additional defects in either locus. *Work supported by the "Associazione E. and E. Rulfo per la Genetica Medica" and by Telethon, Italy (project E.730).*

Program Nr: 1573 from the 1999 ASHG Annual Meeting

Molecular analysis of the P/Q type Ca $^{2+}$ channel a1-subunit gene in patients with progressive cerebellar ataxia. *I. Alonso[1], M.C. Moreira[1], P. Mendonça[1], L. Jardim[2], J. Barros[3], P. Coutinho[4], J. Sequeiros[1], I. Silveira[1].* 1) UnIGENe, IBMC- Univ. Porto, Portugal; 2) Serv. Genética Médica, Hosp. Clínicas Porto Alegre, Brazil; 3) Serv. Neurologia, HGSA, Porto, Portugal; 4) Serv. Neurologia, Hosp. S.Sebastião, Feira, Portugal.

The CACNL1A4 is a brain-specific P/Q type Ca^{2+} channel a1-subunit gene with 47 exons spanning 300 kb. This gene has been implicated in familial hemiplegic migraine (FHM), episodic ataxia type-2 (EA-2), and in a autosomal dominant form of spinocerebellar ataxia (SCA) named SCA6. Six different missense mutations in exons 4, 6, 16, 17 and 36 were found in families with FHM; two EA-2 families have shown mutations in exons 22 and 24 while SCA6 is caused by a $(CAG)_n$ expansion in the 3'-UTR of this channel gene. In order to improve the knowledge about the molecular basis leading to neurodegeneration, we have screened our ataxia families that did not present any of the other known (CAG)n expansions (SCA1-2,7,8, MJD and DRPLA) for CACNL1A a1-subunit mutations. We have ascertained 78 unrelated individuals, representing 26 autosomal dominant families, 9 apparently recessive kindreds and 43 isolated cases; 63 were of Portuguese origin, while 15 were from Brazil. They all have been clinically diagnosed with progressive ataxia with or without other associated features. Polymorphism detection was performed by PCR amplification, single strand conformational polymorphism (SSCP) analysis and sequencing. Expansions of the $(CAG)_n$ repeat were assessed by comparing migratian relative to an M13 sequencing ladder. Mutations in exons 6, 16, 17, 36, 22 and 24 were completely excluded from all our families as well as the presence of a $(CAG)_n$ repeat expansion. In 5 individuals we found a conformational polymorphism in exon 4; we are still sequencing this exon and begun screening the remaining 40 exons. In conclusion, the SCA6 trinucleotide expansion in the CACNL1A4 gene as well as the point mutations already studied are not present among our Portuguese and Brazilian ataxia families. The remaining exons still need to be studied.

Program Nr: 1574 from the 1999 ASHG Annual Meeting

Elucidation of the cDNA sequence and genomic structure of a conserved gene on 7q36 and assessment as a candidate gene for pigmentary glaucoma. *J.S. Andersen[1], E.A. DelBono[1], K. Damji[2], M. Gorin[3], J.S. Schuman[1], C. Mattox[1], J. Haines[4], J.L. Wiggs[1,5].* 1) Dept Ophthalmology, New England Medical Ctr, Boston, MA; 2) University of Ottawa Eye Institute, Ottawa, Ont; 3) University of Pittsburgh, Pittsburgh, PA; 4) Vanderbilt University, TN; 5) Tufts University School of Medicine, Div of Genetics, Boston, MA.

Pigmentary glaucoma is a blinding ocular disorder for which loci have been mapped to 7q36 and 18q. Fine mapping of the critical interval on 7q36 has identified a 1 to 2 cM linked region containing a number of ESTs including SHGC30207, which was derived from a retinal cDNA library and shows similarity to genes of unknown function in C. elegans and Fugu. With expression in the eye and location within the critical interval, this gene is considered a good candidate for pigmentary glaucoma. A combination of database searches, hybridization, and direct sequencing was used to assemble a full-length cDNA. BAC sequencing, inverse PCR, and comparison to genomic sequences was used to elucidate the genomic structure of this gene. A partial cDNA clone was used to generate expression data by Northern blotting and primers were designed to flank the exons for use in screening individuals affected with pigmentary glaucoma. The gene comprises 17 exons spread over 81 kb of genomic DNA. The full-length cDNA is 3.5 kb and encodes a predicted protein of 491 amino acids containing nine predicted transmembrane domains. The putative protein is over 60% similar to the Fugu predicted protein and over 40% similar to that in C. elegans. Expression of the gene in mouse was found in the eye and more strongly in the epididymus and submaxillary gland. Human expression of the gene was also found in the heart, skeletal muscle, liver and kidney. There was no evidence of developmental upregulation at embryonic stages tested. Screening of this gene in affected individuals will allow us to assess this gene in the context of pigmentary glaucoma.

Program Nr: 1575 from the 1999 ASHG Annual Meeting

Familial hypertrophic cardiomyopathy mutations in the regulatory myosin light chain are not necessarily associated with midventricular hypertrophy. *P. Andersen[1], O. Havndrup[2], L.A. Larsen[1], H. Bundgaard[2], J. Vuust[1], K. Kjeldsen[2], M. Christiansen[1]*. 1) Dept Clinical Biochemistry, Statens Serum Inst, Copenhagen, Denmark; 2) Department of Medicine B, The Heart Center, Rigshospitalet, University of Copenhagen, DENMARK.

Familial hypertrophic cardiomyopathy (FHC) is an autosomal dominant heart disease of the cardiac sarcomere. Until now more than 125 mutations have been found in seven different genes encoding sarcomere proteins. Mutations in the two myosin light chains have been characterized to be associated with a special rare type of FHC with midventricular hypertrophy (MVH). In a study of 50 probands with classical FHC and three with MVH FHC we investigated the *MYL2* and *MYL3* genes encoding the regulatory and the essential myosin light chain, respectively. One proband with classical FHC was compound heterozygote for two novel mutations (K102E and IVS6 -1). Another proband had a known A13T mutation. This proband also had the classical form of FHC unlike a previous report where an A13T mutation was associated with a MVH phenotype (Poetter et al., 1997). Only in one of the three probands with MVH we identified a N47K mutation in *MYL2*. We did not identify any *MYL3* mutations among the 53 probands. Concluding the analysis we find that a MVH phenotype is not necessarily associated with a myosin light chain mutation. Reference: Poetter, K. et al. 1997. Nat. Genet. 13:63-69.

Program Nr: 1576 from the 1999 ASHG Annual Meeting

The survival motor neuron (SMN) protein is developmentally regulated and upregulated by NMDA receptor stimulation in cultured cerebellar granule cells. *C. Andreassi[1], A.L. Patrizi[2], M.L. Eboli[1], C. Brahe[2]*. 1) Institute of General Pathology; 2) Institute of Medical Genetics, Catholic University, Rome, Italy.

Spinal muscular atrophy (SMA) is a common autosomal recessive neuromuscular disorder characterized by the degeneration of alpha motor neurons of the spinal cord and weakness of limb and trunk muscles. The disease is caused by absence or mutation in the survival motor neuron (*SMN*) gene. SMN plays an essential role in pre-mRNA splicing and is localized in the cytoplasm and the nucleus where it is concentrated in dot-like structures, termed gems. To investigate whether SMN could be specifically involved in neuronal cell development and function, we studied the expression of SMN in rat cerebellar granule cells during differentiation *in vitro*. Immunofluorescence analysis showed a marked variation in the number of cells with gems during the maturational stages with highest immunoreactivity during the phase of neuronal cell clustering and a marked decrease in nuclear staining in the adult stage of cerebellar neurons. These findings suggest that the appearance of gems is developmentally regulated and support the suggestion that SMN is highly required during the embryo-fetal neuronal development. Since granule cells exhibit a glutamate receptor phenotype similar to that of motor neurons, we also studied SMN expression in response to NMDA receptor stimulation. Exposure to NMDA caused a transient increase in gems and SMN mRNA. To our knowledge, this is the first evidence that SMN expression can be modulated by receptor activation.
Supported by Telethon, Italy, grant 828.
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Program Nr: 1577 from the 1999 ASHG Annual Meeting

Mutation analysis of the GJB2 gene by DGGE in Greek patients with sensorineural deafness. *T. Antoniadi[1], A. Pampanos[2], M. Petersen[1,2]*. 1) Dept Genetics & Molec Biol, Mitera Maternity Hosp, Athens, Greece; 2) Dept Genetics Institute of Child Health, Athens, Greece.

Congenital deafness or hearing impairment affects about 1 in 1000 children and 50% of this hearing loss is genetic. Nonsyndromic recessive deafness accounts for approximately 80% of genetic hearing loss. The first locus defined for recessive deafness, DFNB1, is located on chromosome 13q11. It accounts for a large proportion of cases, due to mutations in the GJB2 gene, which encodes the gap junction protein connexin 26. A major mutation has been identified: a deletion of a G within a stretch of 6 Gs at position 30-35 of the GJB2 cDNA which leads to a premature stop codon at nucleotide 38 and accounts for the vast majority of mutant alleles. In the Greek population we have determined the carrier frequency of this mutation to 3.5%. Since there is still need for identifying the rest of the pathological alleles we developed a DGGE method for screening the entire coding region and surrounding regions. In order to amplify the entire GJB2 coding region we selected the optimal location of primers using the WinMelt program, and GC-clamps were optimized by analyzing the placement effect on the DNA melting profile. The GJB2 cDNA sequence and the surrounding areas (nt -103 to nt 681+320) were divided in 3 amplifiable segments. Using this approach, we started screening 66 chromosomes (33 patients) with prelingual, sensorineural deafness, where syndromic forms and environmental causes of deafness had been excluded. Until now, in addition to 20 alleles with the 35delG mutation (Pampanos et al, abstract this meeting), we have identified 11 candidate mutants in the 5 and 3 segment of the gene, which are currently analyzed by sequencing.

Program Nr: 1578 from the 1999 ASHG Annual Meeting

Progress towards developing a mouse model of Hereditary Haemorrhagic Telangiectasia. *H.M Arthur[1], D.I Wilson[1], E. Torsney[1], G. Renforth[1], A.G. Diamond[2], J. Burn[1].* 1) Human Genetics, University of Newcastle, UK; 2) SMIVS,University of Newcastle, UK.

Endoglin (CD105) is a transmembrane glycoprotein expressed by mammalian endothelial cells. It binds TGFbeta isoforms 1 and 3 and modulates TGFbeta1 signalling. Humans, heterozygous for deleterious mutations in the endoglin gene, develop the disease Hereditary Haemorrhagic Telangiectasia Type 1 (HHT1), which is characterised by bleeding from vascular malformations. In order to study the role of endoglin in vivo and to develop an animal model of HHT1, we have derived mice that carry a targeted nonsense mutation in the endoglin gene. Embryos homozygous for this mutation show severe deficiencies in haematopoiesis, vasculogenesis and angiogenesis in the yolk sac and fail to progress beyond 10.5 days post coitum. This phenotype is remarkably similar to that of both TGFbeta receptor II and TGFbeta1 knockout mice, indicating that endoglin is needed in vivo for TGFbeta1 signalling during formation of the extra-embryonic vasculature. We have also observed abnormal cardiac development in homozygous endoglin-deficient embryos suggesting a particular role for endoglin in cardiogenesis. Recently, we have made significant progress towards the development of an animal model of HHT. Some animals, heterozygous for the endoglin mutation, develop extensive abnormally dilated and weak-walled vessels typical of the vascular lesions seen in HHT patients.

Program Nr: 1579 from the 1999 ASHG Annual Meeting

The Fanconi anemia gene *FANCG*: spectrum of mutations and phenotype correlations. *A.D. Auerbach[1], J. Greenbaum[1], S.D. Batish[1], P.F. Giampietro[2], P.C. Verlander[1]*. 1) Rockefeller Univ; 2) Cornell Medical College., New York, NY.

Fanconi anemia (FA) is a genetically heterogeneous autosomal recessive syndrome associated with chromosomal instability, hypersensitivity to DNA cross-linking agents, and predisposition to malignancy. The gene for FA complementation group G (*FANCG*) has recently been cloned by functional complementation and is identical with human *XRCC9*, which maps to 9p13. The cDNA is predicted to encode a polypeptide of 622 amino acids, with no homology to any known protein that might suggest a function for *FANCG*. We have used SSCP analysis and direct sequencing to screen genomic DNA from a panel of 262 racially and ethnically diverse unrelated FA patients from the International Fanconi Anemia Registry (IFAR) for mutations in *FANCG*. IFAR patients with known mutations in *FANCA* (138 probands) and *FANCC* (53 probands) were excluded. When a sequence variant was identified, additional family members, additional probands of similar ethnicity, and normal individuals were screened to determine if the variant was associated with the FA phenotype. Conservation of sequence in human, mouse and Chinese hamster was also considered in determining pathogenicity. A total of 23 variants were detected, 18 of which are likely to be pathogenic mutations. 43 patients (34 probands) are in FA complementation group G (FA-G), based on the detection of at least one pathogenic mutation in *FANCG*. We estimate that FA-G accounts for ~10% of all FA. The most frequent pathogenic mutations found in the IFAR population are: IVS3+1G>C (Korean/Japanese); IVS8-2A>G (Brazil); IVS11+1G>C (French Canadian); 1184-1194del (N. Eur.); 1794-1803del (N. Eur.). Recurring normal variants include 890C/T (297T/I), 1538G/A (513R/Q) and 1133C/T (378S/L; African Amer.). Most *FANCG* mutations were associated with a severe phenotype, while patients with amino acid substitutions usually displayed a mild phenotype with few major congenital malformations (CM) and a later onset of hematologic abnormalites (HA). Mean number of major CM was 1.8; median age on onset of HA was 4.8 years. Identification of mutant alleles in FA patients will aid in prediction of clinical outcome and in decision-making regarding therapeutic modalities.

Program Nr: 1580 from the 1999 ASHG Annual Meeting

Homozygosity mapping identifies the chromosomal location of a rare congenital contracture gene. *S.J. Bale[1], G.R. Rogers[1], R. Bailey[1], F. Austin[1], J.C. Marini[2], W.A. Cabral[2], J.G. Compton[1], W. Paton[3].* 1) NIAMS, NIH, Bethesda, MD; 2) NICHD, NIH, Bethesda, MD; 3) Alaska Native Med Cntr, Anchorage AK.

The first description of Kuskokwim Syndrome (KS) was published in 1969 (Wright DG), and the first identified case was born in 1922. Patients with this autosomal recessive disease have congenital contractures of large joints and other skeletal anomalies including absent, small, or displaced patellae, spinal and foot defects. In severe cases, knees become fully flexed and patients ambulate by walking on their knees or "duck walking". KS has been reported only in Yup'ik Eskimos of the Yukon-Kuskokwim river delta of SW Alaska. We traveled to 8 Yup'ik villages and 2 towns to ascertain 12 families, including 16 affected KS individuals, 6 probable affecteds, and 47 unaffected 1°relatives. Using homozygosity mapping on pooled DNA from the first 8 families, we evaluated > 300 markers. All 11 affected individuals were homozygous for a single KRT9 allele on 17q12-q21. Further studies revealed a conserved haplotype ("4-1-4") of 3 markers (KRT9-D17S846-D17S800), spanning ~26cM. In the 2nd group of 6 "probable KS" patients, 3 were homozygous 4-1-4, 2 carried one 4-1-4 haplotype and one 4-1-3, and one was heterozygous for the 4-1-4 haplotype but homozygous at D17S846. The 4-1-4 haplotype had a frequency of 92% in affecteds. Analysis of 49 normal controls showed that 4-1-4 occurs with a maximum frequency of 3% in the Yup'ik population. Detailed examinations of 6 KS patients revealed new observations: hx of multiple fractures; horizontal sacrum, superior angulation of transverse processes of lower lumbar vertebrae, highly attenuated patellar/triceps tendons. Clinical features suggest that a gene involved in bone or tendon development may underlie KS. Using radiation hybrids, we are fine mapping COL1A1, which may be a candidate gene for this disorder, and had been located to 17q22. Electrophoresis on SDS-urea-PAGE of type I procollagen from KS fibroblasts showed a slight migration delay of the a1 (I) collagen chain (derived from pro a1(I) by endogenous processing enzymes), suggesting a possible mutation in the C- or N- terminus. Mutation analysis of the N- terminal region was negative.

Program Nr: 1581 from the 1999 ASHG Annual Meeting

Presence of defective and normal LCD40 cDNAs in a patient with X-linked hyper IgM syndrome. *R.M. Barbouche, D.M. Fathallah, M. Bejaoui, Y. Boukhdir, K. Dellagi.* Dept of Immunology, Institute Pasteur de Tunis, Tunisia.

The X-linked form of hyper IgM syndrome is a rare hereditary immunodeficiency characterized by impaired immunoglobulin class switching from IgM to IgG, IgA or IgE. Affected individuals have a defective interaction between the CD40 molecule expressed by B cells and its ligand (LCD40) expressed on the surface of T cells. Several mutations in the LCD40 gene underlying the disease have been reported. The diagnosis of a severe form of X-linked hyperIgM syndrome was established in a Tunisian boy, by serum immunoglobulin measurement and confirmed by the lack of LCD40 expression on the surface of the patient peripheral blood lymphocytes as assessed by flow cytometry analysis using a chimeric human CD40-murin Fc IgG2a recombinant fusion protein. To investigate the molecular basis of the LCD40 expression defect in our patient, we have used specific primers to amplify by RT/PCR the LCD40 cDNA. Experiments have yielded repeatdly two cDNA fragments of different sizes. One with the expected 760bp full length coding region and the second beong approximatively 100 bp shorter. These results were confirmed by several experiments using different sets of primers. DNA sequencing has shown that both fragments were LCD40 cDNA specific. Interestingly, the smaller fragment has an in frame deletion that leads to exon 3 skipping while the sequence of the large fragment was normal. This peculiar pattern of the cDNA expression of an X-linked gene raises different hypothesis among which a possible gene duplication with defective expression, an alternative erroneous splicing or cellular mosaicism. These possibilities are currently under investigation.

Program Nr: 1582 from the 1999 ASHG Annual Meeting

ATM gene expression patterns during early mouse development. *S.G. Becker-Catania[1], H.I. Kornblum[2], R.K. Iyer[1], R.A. Gatti[1]*. 1) Department of Pathology, UCLA School of Medicine, Los Angeles, CA. 90095-1732; 2) Department of Molecular and Medical Pharmacology, UCLA School of Medicine, Los Angeles, CA. 90095.

Ataxia-telangiectasia (A-T) is an autosomal recessive disease characterized by progressive ataxia, telangiectasia, radiosensitivity, increased cancer susceptiblity, and immunodeficiency. The neuropathogenesis of A-T is marked by neuronal loss in the cerebellum (Purkinje cells, granule cells) as well as by demyelination and gliosis in the brain and spinal cord. Extensive immunohistologic studies of patient cerebellums have shown abnormal dendritic arborization and axonal degeneration of the Purkinje cells but have not elucidated the central lesion which leads to the neurodegeneration. ATM knockout mice, although not presenting with ataxia or significant cerebellar changes, have exhibited a loss of dopaminergic neurons in the corpus striatum. We have used in situ hybridization to analyze the expression of the ATM gene in the central nervous system during development, with an emphasis on the midbrain and cerebellar regions. We designed a riboprobe to the mouse atm gene and examined expression at embryonic days 12, 15.5, and 17 in sagittal and coronal sections from C57Bl mouse embryos. At embryonic day 12, we see ubiquitous expression throughout the central nervous system; expression is seen in proliferating neuroepithelial cells lining the ventricles, a subpopulation of which will become the cerebellar primordium as well as the midbrain. Expression in embryonic days 15.5 and 17 are currently being analyzed. The localization of the ATM message in the neuroepithelium may provide insight into which of these neuronal cell precursors may contribute to the ultimate Purkinje cell loss, and which neural stem cell populations would be most appropriate to attempt to replace by neural stem cell transplantation.*in situ*.

Program Nr: 1583 from the 1999 ASHG Annual Meeting

GTP-Cyclohydrolase 1 mutations in autosomal dominant dopamine responsive dystonia and autosomal recessive hyperphenylalaninemia. *H.M. Bedford[1,2], C.A. Rupar[1,2,3], J.M. Gillett[3], R. Casey[4]*. 1) Biochemical Genetics Laboratory, CPRI; 2) Dept Paediatrics; 3) Dept Biochemistry, University of Western Ontario, London, Canada; 4) Dept Paediatrics, University of Saskatchewan, Saskatoon, Canada.

Hereditary progressive dystonia with marked diurnal fluctuation (HPD) or dopa-responsive dystonia (DRD) is an autosomal dominant, childhood onset postural dystonia characterized by diurnal fluctuation and response to dopamine therapy. GTP-cyclohydrolase 1 (GCH1), maps to chromosome 14q and is the first and rate limiting step in the synthesis of tetrahydrobiopterin (BH4). Complete GCH1 deficiency, which is autosomal recessively inherited, results in a rare form of hyperphenylalaninemia. As an approach to understanding how these clinically and genetically disparate disorders could be due to mutations in the same gene, we have characterized causative mutations in a child with HPD/DRD and one with BH4 deficiency hyperphenylalaninemia. Analysis of sequences generated from PCR amplification of the GCH1 promoter and exons including exon/intron junctions revealed a novel point mutation in a sporadic case of HPD/DRD who presented with childhood-onset oromandibular dystonia. The G->A mutation in exon 6 at nucleotide 722 of the coding sequence results in an Arg to Gln substitution at amino acid 241 (Arg241Gln) and the loss of an AvaI restriction enzyme cleavage site. The child with hyperphenylalaninemia is a compound heterozygote for 2 novel mutations; C->T in exon 1 at position 276 resulting in a Leu92Phe substitution and C->G in exon 1 at nucleotide 312 resulting in a Phe104Leu substitution. The parents are heterozygous with no clinical evidence of neurologic sequelae. Neither mutation has been reported in patients with HPD/DRD. Mutations in the GCH1 gene in patients with HPD/DRD are scattered throughout the GCH1 gene with none prevalent. Mutations in the GCH1 gene which result in hyperphenylalaninemia have been described exons 1, 5, and 6 and located closely to DRD mutations. There are no mutations common to HPD/DRD and hyperphenylalaninemia. Establishing genotype/phenotype correlations between these two very distinct disorders will require further analysis of protein structure.

Program Nr: 1584 from the 1999 ASHG Annual Meeting

INVOLVEMENT OF THE HLXB9 GENE IN CURRARINO SYNDROME. *E. Belloni[1], G. Martucciello[3], D. Verderio[1], E. Ponti[1], M. Seri[3], V. Jasonni[3], M. Torre[3], M. Ferrari[1], L.-C. Tsui[2], S.W. Scherer[2].* 1) Unitá di Genetica e Diag Molec, DIBIT-HSR, Milano,Italy; 2) Dept. of Genetics, The Hospital for Sick Children Toronto, ON, Canada; 3) Giannina Gaslini Insitute, University of Genoa, Italy.

Anorectal malformations (ARMs) account for 1/4 of digestive malformations that require neonatal surgery. ARMs have been found in association with sacral anomalies in about 29% of cases. Caudal Regression Syndrome (CRS) is a multisystemic disorder with an overall incidence of 1/7500 live born, presenting total or partial sacrococcygeal agenesis, anorectal malformations, neural changes and urogenital malformations. A peculiar form of CRS is the Currarino Syndrome (CS), characterised by hemisacrum, ARMs and presacral mass (anterior meningocele, teratoma and/or rectal duplication). This syndrome, defined as autosomal dominant, presents high phenotypic variability, but true CS patients always show the typical hemisacrum with intact first sacral vertebra. Recently, the involvement of the HLXB9 homeogene with sacral malformations has been demonstrated: in order to precisely evaluate its role in the development of different sacral phenotypes, we performed Double Gradient-DGGE (DG-DGGE) mutation analysis and DNA sequencing on a total of 27 cases. Patients were divided into 3 groupings, according to strict clinical criteria. Group 1: patients affected by CS; group 2: patients affected by CRS along with ARMs and sacral hypodevelopment; group 3: patients affected by total sacral agenesis along with ARMs. We analysed a total of 1720 bp, including the complete HLXB9 coding region (3 exons) and the intron-exon boundaries. We identified mutations in 4 CS patients (2 missense, 1 frameshift, and 1 splicing). In 3 more CS patients, we identified the presence of deletions of HLXB9, suggesting that haploinsufficiency for this gene is the cause of the disease. We didn't identify any alteration in any of the remaining 17 patients examined. Our results suggest a specific involvement of the HLXB9 homeogene in Currarino Syndrome and possibly not in other sacral malformations.

Program Nr: 1585 from the 1999 ASHG Annual Meeting

A Fibroblast Growth Factor Receptor 3 (FGFR3) Lys650Asn Mutation Causes Hypochondroplasia. *G.A. Bellus[1], P.W. Speiser[2], M.K. Webster[3], D.J. Donoghue[3], C.A. Francomano[4].* 1) Dept Dermatology, B-153, Univ Colorado Health Sci Ctr, Denver, CO; 2) Dept Pediatrics, N Shore Univ Hosp, New York Univ School of Med, Manhasset, NY; 3) Dept Chemistry & Biochemistry, Univ California, San Diego, Lajolla, CA; 4) Medical Genetics Branch, NHGRI, NIH, Bethesda, MD.

Hypochondroplasia is a relatively common autosomal dominant skeletal dysplasia with a phenotype that is similar to, but milder than, achondroplasia. Previous studies have shown that the majority of cases (approximately 50% - 75%) are caused by one of two point mutations (C1620A or C1620G) in the FGFR3 gene resulting in an Asn540Lys substitution in the intracellular tyrosine kinase domain. However, substantial proportions of patients with clinical diagnoses of hypochondroplasia do not have FGFR3 Asn540Lys mutations. Using PCR techniques, we have screened a large group of patients with clinical features of hypochondroplasia who do not have FGFR3 Asn540Lys mutations looking for other FGFR3 mutations. Two patients were found to have lost a *Bbs-1* restriction site in exon 15 and direct sequencing demonstrated that both patients were heterozygous for a G1950T transversion leading to a Lys650Asn substitution. Evaluation of DNA from the parents of these patients revealed that this mutation occurred sporadically in one patient and was inherited from the other patient's father (who also showed skeletal disproportion and mild short stature). These results demonstrate that 3 different amino acid substitutions at the FGFR3 Lys650 codon can result in 3 distinct genetic syndromes. A cDNA construct of this mutation was made and expressed by transient transfection in NIH 3T3 cells. FGFR3 immunoprecipitation and autophosphorylation assays demonstrated the Lys650Asn mutation results in ligand-independent constitutive activation of the FGFR3 tyrosine kinase domain but to a lesser degree than the thanatophoric dysplasia, type 2 FGFR3 Lys650Glu or the SADDAN FGFR3 Lys650Met mutations. These results support the hypothesis that, in FGFR3 related skeletal dysplasias, the severity of the skeletal findings correlates with the level of constitutive FGFR3 tyrosine kinase activity and highlight the importance of the Lys650 codon in FGFR3 function.

Program Nr: 1586 from the 1999 ASHG Annual Meeting

Mutations of the ATPase7B gene in Tunisian patients with Wilson Disease. *M. BenFadhel[1], M.D. Fathallah[1], M. Ben Hariz[1], F. Amri[1], K. Dellagi[1], A. Bobba[2], V. Petrogall[2], E. Marra[2], S. Giannatasio[2].* 1) Immunology, Institute Pasteur Tunis, Tunis, Tunisia; 2) CNR-Centro di Studio sui Mitocondri e Metabolismo Energitico, Bari -Italy.

Wilson disease (WD) is an autosomal recessive genetic disorder characterized by impaired copper transport. Accumulation of the copper in the liver and secondarily in the brain , kidney and cornea results in different clinical manifestations of the disease (hepatic, neurologic or combined form). The gene responsible for WD encodes for a copper transporting P-type ATPase (ATP7B) highly homologous to that of the X-linked copper transport defect : Menkes disease. We have investigated the mutations affecting the ATP7B gene in 8 patients from Tunisia (North Africa) affected by Wilson disease. All these patients were born off first degree consanguineous marriages and belonged to six unrelated families. Four patients had the hepatic form while two had the neurologic one and two had mixed symptoms. We have used SSCP to screen the entire ATP7B coding sequence as well as the exon-intron junctions. The mutations were identified by automated DNA sequencing of the shifted fragments. We characterized five missense mutations, one was already reported (Arg832Lys,exon 10) and the remaining four are newly described (Ser744Pro,exon8;Leu813Ile,exon9;Lys787Ile,Exon9; Val1140Asp, exon16). All the detected mutations affected structurally important regions of the ATP7B molecule which might explain the severe phenotype of our patients. We noticed the absence of the His1069Gln, the most common mutation in European and North Mediterranean population. We have also observed several silent mutations in the ATP7B gene of the patients studied probably associated to polymorphism of the gene in the Tunisian population. Our results suggest that the spectrum of mutations causing Wilson disease in North African populations would be different from the other populations of the northern Mediterranean basin.These data will help providing an early or prenatal diagnosis.

Program Nr: 1587 from the 1999 ASHG Annual Meeting

Mutation screening of the Tuberous Sclerosis gene 1 (TSC1) by protein truncation test. *P. Benit, A. Kara-Mostefa, S. Hadj-Rabia, A. Munnich, J.-P. Bonnefont.* Unité de Recherches sur les Handicaps Génétiques de l'Enfant, INSERM U393, Hôpital Necker, Paris, France.

Tuberous Sclerosis (TSC) is caused by mutations in either the TSC1 gene (9q34) or the TSC2 gene (16p13). More than 90% of TSC1 mutations are predicted to truncate Hamartin, the TSC1 gene product. Taking advantage of these data, we devised a protein truncation test (PTT) to analyze the entire coding sequence of the TSC1 gene in four overlaping fragments. A total of 12 sporadic cases and 3 familial cases linked to 9q34 were studied using a combination of PTT and SSCP analysis. While PTT detected 4/15 mutations (1250+1 G®A, 1473 del C, 1491 del AG, Q743X), SSCP picked up one missense mutation (L72P) and two small deletions (1473 delC, 1491 del AG) but failed to detect two truncating mutations (1250+1 G®A, Q743X) identified by PTT. TSC1 mutation was identified in all three familial cases but in two of the 12 sporadic cases only. Our results emphasize the interest of PTT as a rapid, easy and efficient method for mutation detection in TSC.

Program Nr: 1588 from the 1999 ASHG Annual Meeting

An X-linked T-cell Activation Syndrome characterized by Diarrhea, Respiratory Infections and Autoimmune Disease, harbors rare mutation of the Wiskott-Aldrich Syndrome gene alternate promoter. *C.L Bennett[1], R. Yoshioka[4], H. Kiyosawa[4], D. Barker[3], P. Fain[2], A. Shigeoka[3], I. Blair[1], P.F Chance[1]*. 1) Pediatrics, University of Washington, Seattle, WA 98117; 2) University of Colorado in Denver, Denver, CO 80933; 3) University of Utah, Salt Lake City, UT 84112; 4) Fukushima Medical College, Fukushima-Ken, Japan, 960-12.

We describe an X-linked syndrome with polyendocrinopathy, immune dysfunction & diarrhea (XPID), that commonly results in death during infancy. In this pedigree of 20 affected males, there are two survivors, aged eight and 30 years. Cyclosporin A treatment has produced clinical improvement in diarrhea, and resolution of autoimmune hemolytic anemia and arthritis. Linkage analysis mapped the gene to a 30-cM interval defined by the markers DXS8083 and DXS441 on Xp11.23-11.22, encompassing the gene for Wiskott-Aldrich Syndrome (WAS) and X-linked thrombocytopenia (XLT). The maximum LOD score was 3.81 (theta=0) at DXS255 which is closely linked to WAS. Mutations in the WAS gene have been found in patients with WAS and in patients with the related disorder XLT, indicating that XLT and WAS are allelic variants. To explore the possibility of further allelic heterogeneity in WAS gene mutations, we investigated mutations in XPID. The clinical features of XPID are similar to those of WAS and the XPID gene has been mapped to a region on the X chromosome which contains the WAS locus. Northern blot analysis detected the same relative amount and same-sized WAS gene message in XPID patients and in a normal control. However, in addition to the predicted coding regions we also sequenced the recently identified alternate promoter and untranslated upstream first exon. We identified a C-T transition within the first 8-bp of the alternate translation start site that generates an ATG translation start codon that once spliced into the mRNA transcript, predicts a frameshift mutation. A Nla III restriction fragment length polymorphism generated by this C-T transition segregated with the disease in this family. We propose that the XPID disorder may represent further heterogeneity of clinical manifestations resulting from mutations in the WAS gene.

Program Nr: 1589 from the 1999 ASHG Annual Meeting

CMT1A-REPs based PCR strategies to identify duplications/deletions in Charcot-Marie-Tooth type 1A (CMT1A) and Hereditary Neuropathies with liability to Pressure Palsies (HNPP). *R.B. Bernard[1], A. Navarro[1,2], J. Pouget[3], C. Desnuelle[4], N. Philip[1,2], M. Fontés[2], N. Lévy[1,2]*. 1) Département de Génétique Médicale, Hôpital d'enfants de la Timone, Marseille, France; 2) Inserm U491 "Génétique médicale et Développement" Faculté de Médecine, Marseille, France; 3) Clinique des maladies Neuromusculaires, Hôpital Timone, Marseille, France; 4) Service de reeducation fonctionnelle, Hôpital l'Archet, Nice, France.

CMT1A and HNPP are usually caused by a 1.5 Mb duplication or deletion respectively. These rearrangements occur by unequal meiotic recombination between homologous sequences flanking the 1.5 Mb region (distal and proximal CMT1A-REPs). Reiter et al. (1996), identified a recombination "hotspot" between the CMT1A-REPs and developed a Southern blot approach to detect a 3.2 Kb (EcoRI/SacI) or 1.7 Kb (EcoRI/NsiI) junction fragment in 75% of CMT1A duplicated patients; or a 7.8 Kb (EcoRI/EcoRI) junction fragment in the same proportion of HNPP deleted patients. CMT1A/HNPP diagnosis is technically challenging, time consuming, costly and require isotopic labelled probes. To identify CMT1A duplications, we used a set of primers surrounding EcoRI (distal REP) and SacI (Proximal REP) restriction sites. An EcoRI+NsiI digestion of the PCR products identified a 1.7 Kb junction fragment only in patients for whom duplication resulted from a recombination within the "hotspot". To rapidly detect the HNPP deletions, a PCR assay was designed, using primers flanking the EcoRI and NsiI sites. Prior to the PCR assay, DNAs were digested with EcoRI and NsiI to avoid amplification of the normal REPs. Only the HNPP specific recombinant fragments were amplified. 70 CMT1A and 30 HNPP patients, all presenting a known duplication/deletion by recombination within the "hotspot", were analyzed and identified (100%). In addition, we used a microsatellite analysis to detect the CMT1A duplications/HNPP deletions due to recombination outside the hotspot. Our strategy rapidly identifies almost the entire set of duplications/deletions. It should become a key approach for genetic counselling and prenatal diagnosis of these peripheral neuropathies. Different methods from other groups will be compared.

Program Nr: 1590 from the 1999 ASHG Annual Meeting

Universal Mutation Database (UMD): Software and database for the analysis of mutations in human genes. *C. Béroud[1], G. Collod-ô[1], C. Gallou[1], C. Boileau[1], T. Soussi[2], C. Junien[1].* 1) INSERM U383, Hôpital Necker Enfants malades, PARIS CEDEX 15, FRANCE; 2) Institut Curie, CNRS UMR 218, PARIS CEDEX 05, FRANCE.

With the development of our knowledge of genes alterations involved in human diseases, it has become clear that the identification of mutations plays a critical role not only for diagnosis and prognostic but also for research. The Universal Mutation Database software (UMD) was developed as generic software to create locus specific databases (LSDB). It was developed with the 4th Dimension® package from ACI. It runs on Macintosh or compatible computers and also on IBM PC and compatibles. Since September 1997, it was also used to create the UMD Web site (http://www.umd.necker.fr) which includes today the APC, FBN1, LDLR, MEN1, p53, SUR1, VHL and WT1 LSDB. It has been queried more than 300 000 times. UMD Databases are comprehensive LSDB that contains all reported mutations localized in the coding sequence and splice site mutations of a specific gene. Specific clinical data can also be included depending of each gene/disease (available for FBN1, LDLR, MEN1, SUR1, VHL, WT1). Each data is checked by curators. The UMD software is freely available (ftp.umd.necker.fr) and includes many tools to analyze data. These tools includes routines to: identify preferential mutation sites; indicate mutational events; study the relative distribution of mutations at all sites and sort them according to their frequency; display the distribution of mutations in each exon and evaluate the significance of the differences between observed and expected mutations; search for repetitive sequences involved in the mutational event; study mutated pyrimidine dimers on both strands; study mutations accordingly to their phylogenic conservation ; align repeated domains and compare the distribution of mutations in these domains ; study the mutational event either at the nucleotide or at the amino acid level. A graphical display is available for these routines. The use of optimized searching and sorting tools to select records from any fields gives access to a large range of analyses. They are particularly useful for phenotype/genotype correlation and molecular epidemiology studies.

Program Nr: 1591 from the 1999 ASHG Annual Meeting

Mutational Spectrum of Pallister-Hall Syndrome. *L.G. Biesecker[1], J.M. Graham[2], T. Grebe[3], G. Neri[4], R. Pagon[5], C. Bonneman[5], M. Partington[5], C. Killoran[1].* 1) Genetic Dis Res Branch, LGDR/NHGRI/NIH, Bethesda, MD; 2) UCLA, Cedars-Sinai Medical Center; 3) University of Arizona, Phoenix; 4) Universita Cattolica, Rome, Italy; 5) Other Institutions.

Pallister-Hall syndrome is a human developmental anomaly syndrome that includes hypothalamic hamartoma, polydactyly, bifid epiglottis, and visceral malformations. It is inherited in an autosomal dominant pattern and has been shown to be caused by mutations in the Gli3 zinc finger transcription factor gene. Here we report 11 mutations that cause PHS in addition to the originally described two mutations. These newly reported mutations have been found in sporadic (eight cases) and familial (three cases) occurrences of the disorder. In all cases, the mutation predicts a premature truncation of the GLI3 protein due to 1-19 bp deletions (seven cases), insertions (1 case) or nonsense mutations (three cases). There are no substitutions known to cause PHS. The position of the mutations within the gene ranges from cDNA bp 2146 to 3324, all of which are 3' to the zinc finger encoding domains. These mutations overlap with some truncation mutations that have been associated with apparantly isolated post-axial polydactyly type A (PAP-A). We will review these mutations in light of the molecular models of GLI3 activity and correlate the mutations with the phenotypes of the affected members of the pedigrees.

Program Nr: 1592 from the 1999 ASHG Annual Meeting

Gamma Crystallin and the Congenital Aculeiform Cataract. *G.D. Billingsley[1], M.K. Priston[1], D.F. Schorderet[4], F.L. Munier[4,5], E. Héon[1,2,3].* 1) Eye Research Institute of Canada; 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 Ophtalmique Jules Gonin; Lausanne, Switzerland.

Aculeiform cataract is an autosomal dominant cataract characterized by fiberglass-like or needle-like crystals projecting in different directions. The underlying molecular cause is unknown. A 7 cM disease-gene interval was recently identified on chromosome 2q33-36. Further analyses were performed to identify the underlying genetic defect. Mutational analysis of the gamma-crystallin genes cluster was performed in three unrelated European families by direct sequencing. Direct cycle sequencing of crystallin gamma B, C, D, and E was performed. Observed sequence variations were sequenced in both directions and compared to the sequence of a control population without lens opacification. Several polymorphic sequence changes were identified in gamma B, C, D and E. A missense mutation identified in exon 2 of gamma D cosegregated perfectly with the disease phenotype and was not seen in 210 controls, nor in 44 patients affected with age-related cataracts and 39 patients with sporadic congenital cataracts of various subtypes. The sequence change is likely to impair the folding of the protein.The aculeiform cataract phenotype appears to be due to sequence changes in gamma D crystallin. This is the second association of gamma D crystallin with a congenital cataract phenotype. The identification of cataract-related genes is critical to the better understanding of normal lens development and the biology of cataract formation and progression.

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Program Nr: 1593 from the 1999 ASHG Annual Meeting

Autosomal dominant hyperopia and strabismus in patients from 3-generation family associated with mutation in LIM-domain interacting factor CLIM2 gene. *P. Bitoun[1], E. Semina[2], J. Gaudelus[1], J.C. Murray[2].* 1) Dept Genetique Medicale & Pediatrie, C.H.U Paris-Nord, Hopital Jean Verdier, BONDY, 93143 cedex France; 2) Dpt of Pediatrics & Biological Sciences, U of Iowa, IOWA CITY, IA 52242.

Accomodative esotropia (AE) associated with congenital hyperopia and early strabismus has autosomal dominant transmission in some families (Drack et al; P.Bitoun, unpublished). Early diagnosis is of importance since appropriate correction may help prevent strabismus surgery and since AE often is complicated by secondary glaucoma in the 4th and 5th decade. We identified 5 families with autosomal dominant AE in 2 or 3 generations and performed a mutation search in several genes involved in ocular development which may cause abnormal eye size involved in AE:-PITX2, PITX3, CLIM2 and CLIM1. Screening was performed by SSCP analysis of PCR products obtained with gene-specific primers. The products that showed abnormal mobility of the SSCP gel were sequenced and compared with the normal sequence. An abnormal variant was identified in exon 2 of the CLIM2 gene in a 9 year-old female patient and affected sib with dominant AE from a three-generation family with AE transmitted from father and paternal grand-father. Sequencing identified that this mutation is predicted to cause R231H change in the CLIM2 protein. The mutation affects a highly conserved region of the protein that is immediately adjacent to the LIM- and PITX- interacting domains. Both LIM-domain and PITX genes were shown to be involved in ocular development. Comparison with other CLIM2 proteins isolated from different species identified that this position is occupied by arginine in all known CLIM2 proteins identified for drosophila, chicken, xenopus and mouse. The mutation was not found in 250 control chromosomes. Screening of the rest of the family for segregation of the mutation with the disorder as well as other families with autosomal dominant AE is ongoing. The human CLIM2 gene was localised on 10q24-q25 by Genebridge 4 radiation hybrid panel.

Program Nr: 1594 from the 1999 ASHG Annual Meeting

Clinal and molecular analysis of vitreoretinopathies which map to the proximal long arm of chromosome 5. *G.M. Black[1,2], R. Parveen[1], N. Hart-Holden[1], H.G. Brunner[3], D. McLeod[2].* 1) University Department of Medical Genetics and Regional Genetic Service, St Mary's Hospital, Manchester,UK; 2) University Department of Ophthalmology, Manchester Royal Eye Hospital, Manchester,UK; 3) Department of Human Genetics, Nijmegan University Hospital, Nijmegan, Netherlands.

Wagner syndrome is an autosomal dominant condition which is not associated with systemic stigmata. The ocular findings include an optically empty vitreous, chorioretinal atrophy, cataract and rhegmatogenous retinal detachment secondary to abnormal vitreoretinal adhesion. The condition is heterogeneous and genetic mapping has identified a locus on chromosome 5q13-q14. This region is also implicated in the related condition, erosive vitreoretinopathy which is characterised by abnormal vitreous gel structure, retinal pigment epithelial and electroretinographic abnormalities. We have analysed further families with vitreoretinopathies linked to this region and describe a spectrum of ocular pathologies including defects of early ocular development such as microphthalmia, cataract, persistent hyperplastic primary vitreous, anterior segment dysgenesis and congenital glaucoma. None of the families has extra-ocular manifestations. Genetic mapping presented here in our families reduces the critical region to approximately 2cM and have developed a genomic contig across the region. Subsequent refinement of the physical map allows ordering of known polymorphic microsatellites and excludes CRTL1 as a candidate. CSPG2 lies within the critical region. However mutation analysis of the gene in 15 families reveals no clear evidence that it is the gene underlying WGN1.

Program Nr: 1595 from the 1999 ASHG Annual Meeting

A positional cloning strategy to identify the gene for an autosomal dominant juvenile ALS on chromosome 9q34.

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Amyotrophic lateral sclerosis (ALS) denotes a heterogeneous group of progressive neurological disorders associated with degeneration of motor neurons in the cerebral cortex, brain stem, and spinal cord. Youth onset forms of familial ALS have been described and termed "juvenile ALS". We recently described linkage mapping of a locus for an autosomal dominant juvenile ALS to chromosome 9q34 (ALS4). In order to refine the locus and ultimately identify the disease gene, we have undertaken a positional cloning/candidate strategy utilizing the resources of the Human Genome Project. Additional linkage and recombinant haplotype analysis in a large ALS4 family using a dense collection of microsatellite markers from the originally defined candidate interval has significantly redefined the critical interval to 2-5 Mb. Efforts to establish a complete physical map of the interval are underway. A cosmid contig developed as part of cloning the TSC1 gene is being utilized, in addition to CEPH YAC clones. PAC clones are being identified with view to bridging regions not covered by these clones. Using the "Genemap" EST databases, we have identified over 50 genes in the form of ESTs that localize within the critical interval. Extensive EST sequence contigs have been established for most of these genes by screening EST databases with each mapped EST sequence. Database comparisons with these extended sequences has identified several transcripts whose predicted function suggests that they are candidate genes for ALS4. Expression analysis and fine scale mapping has been used to investigate and prioritize transcripts prior to mutation analysis. This has allowed us to exclude the candidate genes SYT7, GRIN1, STXBPI, and kinesin related gene ATSV. Extensive mutation analysis is underway with several strong candidate genes using the techniques of dideoxy fingerprinting and direct sequencing. Genes under investigation include a homolog of the RING3 gene which has been shown to be induced in neurons undergoing cell death.

Program Nr: 1596 from the 1999 ASHG Annual Meeting

Screening for mutations of *PMP22, MPZ, EGR2, SCIP*and *EGR1*in CMT patients without the CMT1A duplication or *Cx32*mutations. *C.F. Boerkoel, K. Dalakishvili, J.R. Lupski.* Baylor College of Medicine, Department of Molecular and Human Genetics, Houston, TX.

Charcot-Marie-Tooth disease (CMT) is the most common inherited disorder of the peripheral nervous system affecting approximately 1 in 2500 individuals. It is a heterogeneous disorder that has been divided into two major groups: CMT type 1 (CMT1) and CMT type 2 (CMT2). CMT1 is characterized by demyelination with severely reduced motor nerve conduction velocities (NCV); whereas, CMT2 is characterized by axonal loss and normal or mildly reduced NCVs. The CMT1A duplication and *Cx32* mutations account for approximately 70% and 5% of familial and sporadic CMT1 respectively. In the remaining 25% of patients with CMT1, some 80% do not have an identifiable mutation in the coding region of other CMT “disease genes”: *PMP22, MPZ*and *EGR2. EGR2*encodes a transcription factor essential for myelin differentiation. Based on a candidate gene approach, we screened two additional transcription factor genes expressed during myelin differentiation: *SCIP/OCT-6*and *EGR1*. The *SCIP*knockout mouse has severe congenital dysmyelination that improves over time in a manner reminiscent of some forms of human congenital hypomyelination. *EGR1*belongs to the same class of transcription factors as *EGR2*, is expressed in early myelin differentiation, and maps to a locus (5q23-q33) associated with a recessive form of CMT. We report the distribution of mutations among *PMP22, MPZ, EGR2, SCIP*and *EGR1*in more than 100 patients without the CMT1A duplication or *Cx32*mutations.

Program Nr: 1597 from the 1999 ASHG Annual Meeting

Heterogeneous rearrangements of the PLP genomic region in Pelizaeus-Merzbacher Disease: genotype-phenotype correlation in 41 patients. *O. Boespflug-Tanguy[1], G. Giraud[1], C. Mimault[1], V. Isabelle[1], D. Pham Dinh[2], European Network on Brain Dysmyelinating Diseases[1].* 1) Inserm U384, Faculte de Medecine, Clermont-Ferrand, France; 2) UMR CNRS 7624, Universite Pierre et Marie Curie, Paris, France.

Pelizaeus-Merzbacher disease (PMD) is an X-linked developmental defect of brain myelination due to proteolipoprotein (PLP) gene mutations. Using a quantitative fluorescent multiplex PCR technique in 104 PMD affected families, we shown that duplication of the entire PLP gene is the most frequent mutation accounting for 50% of all PMD affected families and 64% of all PLP identified mutations. Furthermore, we found a large PLP gene deletion in a spastic paraplegia 2 family. Clinical evaluation of the 84 PLP-duplicated patients revealed a large range of disease severity with patients able to walk with support in the mildest form to patients with no motor achievment in the most severe form. An intrafamilial homogeneity was observed in the 14 familial forms analysed. The extend of the genomic duplication was further determined in 41 families by using a multiplex PCR with 10 pairs of primers chosen along the Xq22 region encompassing the PLP locus and by pulse field gel electrophoresis. Size of the duplications were greatly variable (40kb<to>1000kb) and not correlated to the clinical severity of the disaese. The two patients with the most severe form had PCR ratio compatible with a PLP triplication. For two patients the telomeric end of the duplication was localised inside the 20 kb end of the cosmid cV698D2 containing the PLP gene. Southern blot and sequencing analysis of the two breakpoint regions have demonstrated distinct rearrangements. Finally in PLP-related diseases, severity is correlated to the number of PLP copies and not to the size of the duplication. Existence of both duplication and deletion events associated with a large male mutation imbalance for duplications (C. Mimault, in press) suggest a mechanism of unequal sister chromatid exchange, as in the demyelinating neuropathies related to PMP22 mutations (CMT1 and HNPP). However, the large diversity found in the break points of PLP duplications suggests a more complex mechanism leading to the great genomic instability of the xq22 region.

Program Nr: 1598 from the 1999 ASHG Annual Meeting

Identification of complex genetic rearrangements in the iduronate-2-sulfatase (IDS) gene causing the Hunter syndrome (MPSII). *M-L. Bondeson[1], S.L. Karsten[1], B-M. Carlberg[1], W.J. Kleijer[2], K. Lagerstedt[1]*. 1) Dept of Genetics and Pathology, Uppsala University, Uppsala, Sweden; 2) Dept of Clinical Genetics, Erasmus University, Rotterdam, The Netherlands.

The Hunter syndrome (or mucopolysaccharidosis type II) is an X-linked recessive lysosomal storage disorder caused by a deficiency in the enzyme iduronate-2-sulfatase (IDS). Clinically, a wide spectrum of phenotypes has been observed ranging from severe to mild. The IDS locus is localised at the boundary between Xq27.3 and q28 and several novel genes have been found in the proximity of the IDS gene including an IDS-related gene with a pseudo-gene like structure (IDS-2). A large number of mutations have been identified as the primary genetic defect causing the Hunter syndrome. Most of the mutations represent point mutations or minor alterations. However, in about 20% of the patients major structural aberrations such as deletions of the entire gene or rearrangements have been identified. In order to gain insights into the mechanisms generating these structural aberrations we have analysed two unrelated patients by Southern blot analysis, PCR and DNA sequencing. In the first patient, an intragenic deletion of exons 5 and 6 in the IDS gene was identified. Sequencing of the deletion junction revealed a complex genetic rearrangement involving duplications and inversions. The second patient suffer from a large deletion of 43.6 kb spanning exons 1-7 of the IDS gene, the IDS-2 locus and exons 3-5 of the gene W. An interesting finding was the identification of a small deletion consensus motif, TG(A/G)(A/G)(G/T)(A/C), in both the 5' and 3' donor sequences. This motif is very similar to motifs found in the immunoglobulin switch regions and also to putative DNA polymerase alpha arrest sites. The location of this consensus sequence in both the 5' and 3' donor sequences spanning the deletion junction, strongly suggests that it contributes to the mutational process resulting in a large deletion. Based on DNA sequence analysis of the two deletions described here, we propose models for how the deletions might be generated by illegitimate recombination during the meiosis.

Program Nr: 1599 from the 1999 ASHG Annual Meeting

The Yemenite deaf-blind hypopigmentation syndrome revisited: SOX10 dysfunction causes different neurocristopathies. *N. Bondurand[1], K. Kuhlbrodt[2], V. Pingault[1], J. Enderich[2], M. Sajus[1], N. Tommerup[3], M. Warburg*[3], R.CM. Hennekam[4], AP. Read[5], M. Wegner[2], M. Goossens[1]*. 1) Inserm U468,hopital Henri Mondor, Creteil, France; 2) Zentrum fur Molekulare Neurobiologie, Universitat Hamburg, Germany; 3) Department of Medical Genetics, Institute of Medical Biochemistry and Genetics, Panum Institute, University of Copenhagen, Denmark *Center for Disabled Children, The Eye Clinic, Copenhagen, Gentofte, Denmark; 4) Department of Pediatrics and Clinical Genetics, AMC, Amsterdam, The Netherlands; 5) University Department of Medical Genetics, St Mary's Hospital, Manchester M13, UK.

Neurocristopathies are disorders characterized by a defect of neural crest development. We previously showed that heterozygous mutations in the sry-related *SOX10* gene are encountered in some cases of Shah-Waardenburg syndrome, a neurocristopathy characterized by the association of Hirschsprung disease (intestinal aganglionosis) and Waardenburg syndrome (pigmentation defects and sensorineural deafness). Moreover, *Sox10*/*SOX10* expression in rodents and human is consistent with a function in neural crest development. We therefore extended the mutation screening to other neurocristopathies. The Yemenite deaf-blind hypopigmentation syndrome was first observed in a Yemenite sister and brother showing cutaneous hypopigmented and hyperpigmented spots and patches, microcornea, coloboma, and severe hearing loss. A second case, observed in a girl with similar skin symptoms and hearing loss but without microcornea or coloboma, was reported as a mild form of this syndrome. Here we show that a *SOX10* missense mutation is responsible for the mild form, resulting in a loss of DNA binding of this transcription factor. In contrast, no *SOX10* alteration could be found in the other, severe case of the Yemenite deaf-blind hypopigmentation syndrome. Based on genetic, clinical, molecular and functional data, we suggest that these two cases represent two different syndromes. These results show that *SOX10* mutations cause various types of neurocristopathies.

Program Nr: 1600 from the 1999 ASHG Annual Meeting

Mutations in the RP1 gene causing autosomal dominant retinitis pigmentosa. *S.J. Bowne[1], S.P. Daiger[1], C.F. Inglehearn[2], M.M. Sohocki[1], K.A. Malone[1], J.R. Heckenlively[3], D.R. Birch[4], S.S. Bhattacharya[5], A. Bird[5], M.M. Hims[2], A.B. McKie[2], L.S. Sullivan[1]*. 1) Human Genetics Ctr, The University of Texas-Houston; 2) Leeds University, Leeds, UK; 3) Jules Stein Eye Institute, UCLA; 4) Retina Foundation of the Southwest, Dallas; 5) Institute of Ophthalmology, London, UK.

Recently we identified the gene responsible for the RP1 form of autosomal dominant retinitis pigmentosa (adRP) and found two different nonsense mutations in 3 families previously mapped to chromosome 8q. The RP1 gene encodes a protein 2,156 amino acids in length, but is comprised of four exons only. In order to determine the frequency and range of mutations in RP1 we screened probands from 56 large adRP families for mutations in the entire gene. After preliminary results indicated that mutations seem to cluster in a 442-nucleotide segment of exon 4, an additional 603 probands with a wide range of degenerative retinal diseases were tested for mutations in this region alone. In addition to the two nonsense mutations originally identified in RP1, we have now identified 7 more disease-associated mutations in 17 unrelated families and two rare missense changes in two additional families. All of the disease-associated mutations lead to a severely truncated protein. We also identified one new polymorphic amino acid substitution, one silent substitution and a rare variant in the 5' untranslated region that is not associated with disease. Based on this study, mutations in RP1 appear to cause 5 to 10% of adRP. A 5bp deletion of nucleotides 2383-2387 and the Arg677stop mutation account for over 50% of these mutations. Further studies will determine if missense changes in certain regions of the RP1 gene are associated with disease and if the background variation on either the mutated or wildtype RP1 allele plays a role in the disease phenotype.

Supported by NEI-NIH grant EY07142 and grants from the Foundation Fighting Blindness, the Hermann Eye Fund, the British RP Society and the Wellcome Trust.

Program Nr: 1601 from the 1999 ASHG Annual Meeting

Incomplete X-linked congenital stationary night blindness: Clinical variability among patients with the L1045insC founder mutation in *CACNA1F* and the search for genetic modifiers. *K.M. Boycott[1], W.G. Pearce[2], N.T. Bech-Hansen[1]*. 1) Department of Medical Genetics, University of Calgary, Calgary, AB, Canada; 2) Department of Ophthalmology, University of Alberta, Edmonton, AB, Canada.

Incomplete X-linked congenital stationary night blindness (iCSNB) is a non-progressive retinal disorder characterized by impaired night vision, decreased visual acuity, myopia, nystagmus, and strabismus. Mutation analysis of the *CACNA1F* gene, which codes for a retina-specific L-type calcium channel a_1-subunit protein (a_1F), in patients with iCSNB has identified a total of 17 different mutations seen in 36 families. One of these mutations, L1045insC, is seen in 15 families of Mennonite ancestry from Western Canada. Clinical variability was examined in 66 patients from these families in terms of night blindness, visual acuity, myopia, nystagmus, and strabismus. In 80% of the patients at least one of the main features of CSNB (night blindness, myopia, nystagmus) was absent. The only clinical feature present in all 66 patients was impaired visual acuity. The variability seen in clinical expression among these patients who share the L1045insC mutation suggests the presence of genetic modifiers.

There is increasing evidence that the manifestations of many genetic disorders are influenced by modifying genes distinct from the disease locus. Of the 66 iCSNB patients examined, 40% have nystagmus as one of the manifestations of the disease. Loci in Xq26, 6p12, and Xp11.4 identified as playing a role in the pathophysiology of congenital motor nystagmus were evaulated as candidate regions for modification of the nystagmus phenotype in iCSNB, and excluded as having a major effect. Similar investigations are currently being performed using candidate regions implicated in the pathophysiology of myopia. Identification of genetic modifiers that affect clinical expression in iCSNB will be important for understanding the disease process and may provide attractive targets for therapy.

This research was supported in part by the RP Research Foundation (Canada), the Alberta Heritage Foundation for Medical Research and the Roy Allen Endowment.

Program Nr: 1602 from the 1999 ASHG Annual Meeting

Detection of the survival motor neuron (*SMN*) genes by FISH: further evidence for a role of *SMN2* in the modulation of disease severity in SMA patients. *C. Brahe, T. Vitali, V. Sossi, S. Zappata, F. Tiziano, G. Neri.* Institute of Medical Genetics, Catholic University, Rome, Italy.

Spinal muscular atrophy (SMA) is a common autosomal recessive neuromuscular disorder characterized by the degeneration of spinal cord anterior horn cells and weakness of limb and trunk muscles. On the basis of age of onset and severity of the clinical course, three forms of childhood-onset SMA (types I-III) and one adult-onset form (type IV) can be distinguished. SMA is caused by the homozygous absence of the survival motor neuron (*SMN1*) gene due to deletion, gene conversion or mutation. *SMN1* and a nearly identical copy (*SMN2*) are located in a duplicated region at 5q13 and encode identical proteins.

The genetic basis for the clinical variability of SMA remains unclear. Data obtained by quantitative PCR assays have previously suggested a correlation between the number of *SMN2* gene copies and disease severity. However, the exact number of genes is difficult to estimate by this method. We have developed fluorescence in situ hybridization (FISH) conditions suitable for visualizing the *SMN* genes. We used as probe a mixture of PCR amplified DNA fragments specific of the *SMN* gene together with a reference probe, located outside of the duplicated SMA region, in order to establish the phase of the cell cycle in interphase nuclei. All patients had at least two *SMN2* genes suggesting that the presence of a single *SMN2* gene is very rare. We found 2 or 3 copies of *SMN2* in type I patients, 3 copies in type II and 3 to 4 copies in type III patients. One individual with very mild adult-onset SMA had 8 copies of *SMN2*. No alterations of the genes were detected by Southern blot and sequence analysis suggesting that all gene copies of *SMN2* were intact. These data provide further evidence that the *SMN2* genes modulate the disease severity but also supports the suggestion that not all *SMN2* genes are functionally equivalent.

Supported by Telethon, Italy, grant 828.

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Program Nr: 1603 from the 1999 ASHG Annual Meeting

Mutation analysis of PEX7 in patients with rhizomelic chondrodysplasia punctata (RCDP). *N.E. Braverman[1], P. Lin[2], G. Steel[2], C. Obie[2], H. Moser[3], A. Moser[3], D. Valle[1,2].* 1) Dept Pediatrics, Johns Hopkins University, Baltimore, MD; 2) Howard Hughes Medical Institute, Baltimore, MD; 3) Kennedy Krieger Institute, Baltimore, MD.

RCDP is a peroxisome biogenesis disorder characterized by rhizomelia, epiphyseal calcifications, cataracts and profound growth and developmental delay. Although most patients have this classical phenotype, milder variants are recognized. We have previously shown that RCDP is caused by mutations in PEX7, which encodes the receptor (Pex7p) for peroxisomal matrix proteins containing a PTS2 targeting sequence. Pex7p is a WD40 repeat protein. We have now completed a mutation survey in 51 RCDP patients. Using SSCP we found 17 unique alleles, accounting for 88%. There 14 missense (6 unique), 56 nonsense (7 unique, 50 are L292X), 18 splice site changes (3 unique, 14 are IVS9+1G>C), 1 deletion and 1 insertion allele. We performed ASO analysis for most mutations; none are population polymorphisms. Using 5 polymorphic markers in the PEX7 gene we show that L292X originated on a single chromosome in the Caucasian population. Preliminary evidence shows that the frequent IVS9+1G>C allele arose several times on different haplotypes.

Suprisingly, overexpression of exon 1 missense alleles (S25F, H39P and G41V) in RCDP null cells revealed they could mediate PTS2 protein import. Molecular modeling of Pex7p on the crystal structure of another WD40 repeat protein, b transducin, reveals that these changes are located in the coiled-coil N-terminus, which is not involved in the b-propeller structure. Of 6 patients with mild phenotypes, one, who has cataracts only, has an N-terminal missense mutation. Another, who has an adult Refsum disease phenotype, likely generates a Pex7p lacking an internal WD40 repeat. Our mild variants have high residual plasmalogen levels, but phytanoyl-CoA alpha-hydroxylase activity remains low. These results indicate that PEX7 alleles with residual function are associated with phenotypes outside the familiar and that specific changes in the b-propeller structure or N-terminus may influence PTS2 ligand binding or transport.

Program Nr: 1604 from the 1999 ASHG Annual Meeting

Tenascin-X (TN-X) deficiency in five patients with Ehlers-Danlos Syndrome (EDS). *S.M. Burridge[1], J. Schalkwijk[2], G. Taylor[1], P.M. Steijlen[2], W.L. Miller[1], J. Bristow[1].* 1) Pediatrics, UCSF, San Francisco, CA; 2) Dermatology, University Hospital, Nijmegen, Netherlands.

The tenascin family of extracellular matrix proteins consists of three members named TN-C (cytoactin, tenascin), TN-R (restrictin) and TN-X. Studies of the expression of TN-C and TN-R suggest important functions during development but TN-C and TN-R knockout mice have minimal neurological abnormalities and no other apparent phenotype. TN-X is associated with collagen fibrils and is highly expressed in connective tissues, but its function is unclear. The TN-X gene overlaps the CYP21 gene for steroid 21-hydroxylase that causes congenital adrenal hyperplasia (CAH) on the opposite strand of DNA on chrom 6p21.3. We previously reported a patient with a contiguous gene syndrome of EDS and CAH who was heterozygous for a 30kb deletion which deletes CYP21 and part of TN-X. We now report the identification of a similar unrelated patient with this contiguous gene syndrome. As in the index case, TN-X is absent from western blots of fibroblast conditioned medium while collagen V secretion is normal. Also like the index case, this patient is heterozygous for the CYP21/TN-X deletion. Protein truncation tests confirmed the presence of this deletion. Both patients appear to carry as yet unidentified inactivating mutations on their second TN-X alleles. This finding suggested that inactivating TN-X mutations might be found without mutations in CYP21 and that TN-X deficiency could be a cause of EDS independant of CAH. We have now identified 3 such patients with mild classical EDS and complete TN-X deficiency. All have typical skin findings, and hyperextensible joints. One patient has spina bifida, one has a dilated aortic root, and one has poorly characterized neuromuscular disease. PCR showed that only one isolated EDS patient carries the 30kb deletion, and he is heterozygous. Microsatellite analysis suggests that the 30kb deletion has occured independently in each patient. This work supports our hypothesis that TN-X deficiency can cause classical EDS in patients who do not also have CAH. Mutational analysis to identify inactivating mutations in non-deleted TN-X alleles is now underway.

Program Nr: 1605 from the 1999 ASHG Annual Meeting

X chromosome inactivation studies in families with Prader-Willi syndrome. *M.G. Butler, J.D. Eskew, L.D. Wilcox, P.K. Rogan.* Section Medical Genetics, Children's Mercy Hosp, Kansas City, MO.

In normal human females one of the two X chromosomes in each somatic cell becomes inactivated in early embryonic development. The inactivation process is thought to be random in any particular somatic cell. However, a subset of female biased X inactivation may occur leading to preferential methylation of X linked genes on either the paternal or maternal X chromosome, for example, in X chromosome rearrangements and certain X linked diseases. Nonrandom X inactivation is increased in fetuses and newborns that are associated with confined placental mosaicism of an autosomal trisomy. This could conceivably result from a reduction in the size of the early embryonic cell pool due to poor growth or selection against trisomic cells. Extremely skewed X inactivation patterns have been reported in Prader-Willi syndrome (PWS) females with upd(15)mat (Lau et al., 1997), which frequently results from postzygotic missegregation in a trisomic conceptus. X inactivation status was assayed using methylation-specific polymerase chain reaction of a polymorphic sequence in the androgen receptor gene (Kubota et al., 1999). We have extended this observation by evaluating PWS families and have found 4 of 13 upd(15)mat females with significant X inactivation, i.e., close to 100% inactivation of one particular allele. Two female individuals and their mothers appeared to show identical X inactivation patterns, consistent with the possibility of hereditary X inactivation in these individuals. However, we also found skewed methylation of the maternally derived allele in one of three deletion PWS females and her mother. As an additional control, we evaluated the pattern of X inactivation in 13 different tissues from a 20-week gestation non-PWS female fetus, which showed tissue-specific skewing of X chromosome methylation. Biased X inactivation may not be more common in subjects with upd(15)mat than in unaffected individuals; however, additional studies are warranted.

Program Nr: 1606 from the 1999 ASHG Annual Meeting

Cell-specific processing of mutant type I collagen in three probands with Osteogenesis Imperfecta. *W.A. Cabral, J.C. Marini.* Sec Connective Tissue Disorder, NICHD/NIH, Bethesda, MD.

In previous comparisons of collagen synthesis by OI osteoblasts and fibroblasts, we reported that the proportion of overmodified alpha chains, as well as their relative electrophoretic delay, was greater in osteoblasts than fibroblasts (Hum Mut 11:395(1998)). Further comparisons of mutant collagen metabolism were conducted in paired primary osteoblasts and fibroblasts to determine the bases of the differences in cell-specific processing. For intracellular processing and secretion studies, we selected cases of non-lethal OI in which normal and mutant type I chains from fibroblasts and osteoblasts were electrophoretically well-resolved. Mutations were identified using RNase A digestion of RNA hybrids, then subcloning and sequencing. We identified point mutations resulting in glycine substitutions: a2(I) gly703®arg (GGA®AGA) and gly922®ser (GGT®AGT), and a1(I) gly997®ser (GGT®AGT). For each proband, the products of steady-state and continuous pulse labelling of osteoblast and fibroblast collagen were compared electrophoretically. For steady-state labelling, the intracellular and secreted proportion of electrophoretically delayed alpha chains was greater in osteoblasts than fibroblasts. Continuous pulse-labelling showed overmodified chains appear rapidly in osteoblasts, about 2 hours sooner than in fibroblasts. Overmodified collagen chains are present in higher proportion and with greater electrophoretic delay than in fibroblasts. Further, the overmodified chains in osteoblast samples have a gradually increasing electrophoretic delay with time, attaining the steady-state pattern in about 4 hours. The proportion of secreted mutant chains was greater in osteoblasts than fibroblasts, where only a low proportion of overmodified form is detected in media samples. In these OI cases, osteoblasts are more permissive than fibroblasts for intracellular survival and secretion of mutant collagen. Although OI is a generalized disorder of connective tissue, the predominant symptoms occur in bone. Cell-specific differences between fibroblasts and osteoblasts in processing and secretion of collagen may be a crucial part of the pathophysiology of OI.

Program Nr: 1607 from the 1999 ASHG Annual Meeting

A newly identified transcript and SPG7, the spastic paraplegia gene, are homologous and are separated by 500kb at 16q24.3. *D.F. Callen[1], S.A. Whitmore[1], M. d'Apolito[2], A. Savoia[2], A. Gardner[1], G. Kremmidiotis[1].* 1) Dept Cytogenetics/Molec Gen, Women's and Children's Hosp, South Australia, Australia; 2) Servizio di Genetica Medica, IRCCS-Ospedale CSS, San Giovanni, Italy.

The gene, SPG7, has recently been shown to be mutated in a recessive form of spastic paraplegia (Casari et al 1998). Exon trapping analyses of cosmid clones 500kb distal to the SPG7 gene on chromosome 16 indicated the presence of a homologous transcript. However, analysis of human EST clones revealed a transcript without a clear open reading frame (ORF). Complete genomic sequencing of 70kb in the region showed the presence of a complex intronic repeat which resulted in mis-alignment and mis-identification of human ESTs. Mouse ESTs which were identified in the region allowed the exons and the ORF of the human gene to be clearly delineated and this was confirmed by RT-PCR experiments. BLASTP searches show that the predicted protein is highly homologous to yeast mitochondrial ATPases as is Paraplegin which is the protein mutated in recessive spastic paraplegia. The newly characterised gene shows 52% identity to Paraplegin. We hypothesise that the close physical proximity is consistent with a duplication event and that this newly identified transcript may be mutated in some patients with this disease.

Program Nr: 1608 from the 1999 ASHG Annual Meeting

Identification of Survival Motor Neuron-interacting proteins, including a novel RNA helicase, by yeast-two-hybrid screening. *L. Campbell, K. Hunter, P. Mohaghegh, Y. Chan, K.E. Davies.* Department of Human Anatomy and Genetics, University of Oxford, Oxford, UK.

Spinal muscular atrophy (SMA) is a common neurodegenerative disease in which reduced levels of the survival motor neuron (SMN) protein results in loss of spinal motor neurons and associated proximal muscle weakness. The SMN protein is ubiquitously expressed. The high levels of SMN normally present in the motor neurons are greatly reduced in type I SMA patients. Proteins interacting with SMN have been identified in previously reported yeast-two-hybrid screens using HeLa and thymus cell cDNA libraries (1,2).

We screened a mouse brain cDNA library in an attempt to identify protein interactions relevant to the specific nature of the motor neuron degeneration characteristic of SMA. As previously reported by other investigators, interactions with prey clones encoding SMN (itself) and SIP1 were identified (1,3). Several additional interactors were also identified, two of which (LK1 and LK2) encoded previously unidentified genes. A consensus sequence for LK1 was obtained from 10 independent clones. Northern analysis showed that LK1 encodes for an mRNA of approximately 6kb, which is highly expressed in muscle and kidney. LK2 (5 clones) encodes a 3kb mRNA which is ubiquitously expressed, with highest levels in testis. Developmental mouse northern analysis indicated high levels of expression as early as embryonic day 7. Database analysis of the coding sequence identified LK2 as a novel member of the RNA helicase family. The significance of this interaction is as yet unknown, but such helicase proteins participate in most cellular RNA metabolic processes, including splicing, in which SMN plays an important role (4).

In vitro co-localisation studies and characterisation of LK1 and LK2 specific antibodies are in progress.

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Program Nr: 1609 from the 1999 ASHG Annual Meeting

Identification and characterization of a candidate gene highly expressed in hippocampus for X-Linked Mental Retardation in Xp22.1-21.3. *A. Carrie[1], L. Jun[2], T. Bienvenu[1], M.C. Vinet[1], S. Frints[2, 3], B. Hamel[4], C. Moraine[5], J.P. Fryns[3], C. Beldjord[1], P. Marynen[2], J. Chelly[1].* 1) INSERM U129, ICGM, Paris, France; 2) The Flanders Interuniversity Institute for Biotechnology, UZ Gasthuiberg, Leuven, Belgium; 3) Center for Human Genetics, Clinical Genetics University, UZ Gasthuiberg, Leuven, Belgium; 4) University Hospital Nijmegen, Department of Human Genetics, Nijmegen, The Netherlands; 5) Centre Hospitalier de Tours, service de Genetique, Hopital Bretonneau, Tours, Franceé.

The Xp22.1-21.3 region, distal to the DMD gene is a region of interest for non specific X-linked mental retardation (XLMR). A confirmation of a potential XLMR locus was obtained through the detection of two inherited overlapping microdeletions associated exclusively with nonspecific mental retardation. In order to refine the MRX gene locus, we constructed cosmid and PAC contigs encompassing approximately 550 kb and covering the smallest deletion. This PAC contig was used by the Sanger Centre (Cambridge, UK) for large scale sequencing of the critical region. With the availability of the genomic sequence, we combined the computational sequence analysis with the screening of cDNA libraries, 5 and 3 rapid amplification of cDNA ends-PCR (RACE-PCR) and RT-PCR procedures to isolate and characterise a unique candidate gene which maps within the critical region. The transcript produced by this gene is highly expressed in the hippocampus including the dentate gyrus, the primary olfactory cortex, the entorhinal cortex, the perirhinal cortex, and occipito-parietal cortices. The combination of mapping data and its particular expression pattern strongly implicate this gene as a candidate for mental retardation in the Xp22.1-21.3 region. Mutations screening in mentaly retarded families which maps in the region and validation of this gene are in progress.

Program Nr: 1610 from the 1999 ASHG Annual Meeting

An in frame deletion of BRCA1 exon 20 in a family with early onset breast cancer and ovarian cancer. *N. Carson, C. Gilpin, A. Hunter, J. Allanson, H. Aubry.* Dept of Genetics, Children's Hospital of Eastern Ontario, Ottawa, ON, Canada.

The BRCA1 gene was cloned in 1994 and since then over 300 different mutations resulting in an increased risk of developing breast and ovarian cancer have been described. Most of these mutations are frameshift or nonsense mutations however a number are missense mutations. Recently large genomic rearrangements have also been characterized. All rearrangements described to date have been out of frame alterations leading to premature stop codons. We describe a family with early onset breast and ovarian cancer that has an in frame deletion of exon 20.

The proband developed breast cancer at the age of 40. She has one sister who developed breast cancer at 34 and another who died of breast cancer which developed at 44 years of age. Their mother died of ovarian cancer. Analysis of RNA using the protein truncation test for the region defined by exons 14 to 24 of the BRCA1 gene revealed a truncated protein approximately 4 kDa shorter than normal suggesting a mutation at the end of exon 23 or in exon 24. Sequence analysis of the cDNA revealed a deletion of all of exon 20 suggesting a possible splice site mutation, however, no mutation could be identified. Southern blot analysis of genomic DNA probed with exon 14- 24 cDNA revealed junction fragments for both HindIII and EcoRI digests while the BglII digest showed reduced intensity for the exon 20 specific fragment. These results support the presence of a large genomic deletion encompassing all of exon 20. Long range PCR using primers that flank the deleted region has shown that the deletion is approximately 4 kb in size.

This rearrangement is of interest as it is an in frame deletion resulting in the loss of 28 amino acids from the carboxyl end of the BRCA1 protein. In order to provide evidence for this mutation being the cause of breast cancer in this family, DNA will be analysed from the proband's affected relatives. Assuming this deletion segregates with breast cancer in this family, this is the first example of a large in frame deletion of BRCA1 leading to the development of breast and ovarian cancer.

Program Nr: 1611 from the 1999 ASHG Annual Meeting

Laron dwarfism mutation in a Chilean family with a probable Mediterranean ancestor. *M.P. Carvallo[1], C. Espinosa[1], M. Sjoberg[1], A. Rodriguez[1], T. Salazar[2], F. Cassorla[2], V. Mericq[2]*. 1) Human Genetics Program, ICBM, Faculty of Medicine, Universidad de Chile; 2) IDIMI,Universidad de Chile.Santiago, Chile.

Laron-type dwarfism is a rare autosomal recessive disorder caused by an insensibility to growth hormone. Several mutations in the growth hormone receptor gene (GHR), involving splice site, nonsense, and missense mutations have been described. One of these mutations creates a new splice site in exon 6 (E180splice), and has been detected in a cohort of 55 patients from south Ecuador and one oriental Jewish patient. It has been shown that this mutation is associated with a specific haplotype (I) within the GHR gene, which is also the most frequent Mediterranean haplotype (52%), suggesting a common Mediterranean ancestor for all these patients. We studied a Chilean family in which the parents were consanguineous and two of the seven children were affected from Laron syndrome. This family lives in the south of Chile in a region where a high aborigine admixture has been detected. We studied all family members through clinical and molecular analysis. Through SSCP and sequence analysis we found the same mutation described above, E180splice, in both patients. Both parents were heterozygous for this mutation, as well as four non affected siblings. Interestingly heterozygous members of the family show normal values in the clinical and phenotype evaluation. Only homozygous affected members have IGF-1 and its respective binding protein IGFBP-3 levels below normal, as well as low stature (-5.5 and -4.7 SDS). Since this is the third time that this mutation has been detected in world patients, we found interesting to perform the haplotype analysis in this family. We found an association of haplotype I with the mutated locus in the family, being this haplotype heterozygous in both parents. The frequency determination of the seven haplotypes in a Chilean population (60 chromosomes) revealed a very low frequency for haplotype I (0.07) being haplotypes II, III and IV, the most frequent ones (0.6, 0.16 and 0.22. These results strongly support the hypothesis that the E180splice mutation in the GHR receptor has a common Mediterranean ancestor. (supported by FONDECYT 197-0276).

Program Nr: 1612 from the 1999 ASHG Annual Meeting

Yeast and mouse models of Friedreich's ataxia. *P. Cavadini[1], S.S. Branda[1], C. Lesnick[1], F. Taroni[2], J. van Deursen[1], G. Isaya[1]*. 1) Pediatric & Adolescent Medicine, Mayo Clinic and Foundation, Rochester, MN; 2) Unit of Cellular Pathology, Department of Neurobiology, Istituto Nazionale Neurologico "C. Besta", Milan, Italy.

Friedreich`s ataxia (FRDA) is an autosomal recessive neurodegenerative disease caused by deficiency of the mitochondrial protein frataxin. Most patients are homozygous for GAA trinucleotide repeat expansions in the first intron of the frataxin gene, while ~4% of cases are compound heterozygous for a GAA expansion and a point mutation. The GAA expansion has been shown to lead to frataxin deficiency which in turn results in mitochondrial iron overload, but the functional consequences of the point mutations remain undefined. To carry out a mutational analysis of frataxin, we have expressed the human protein in a S. cerevisiae strain (yfh1D) lacking the endogenous yeast frataxin homologue (Yfh1p). In this system, the human frataxin precursor is efficiently imported and processed via an intermediate to the mature form. In vitro processing assays show that both cleavages are carried out by the yeast mitochondrial processing peptidase (MPP) and that the mature form of human frataxin is produced by cleavage at or near valine 55. The processed protein restores the respiratory competence of yfh1D indicating that the yeast mitochondrial protein import machinery produces a functional form of human frataxin. Yeast therefore represents an expression system suitable to study the functional consequences of point mutations identified in FRDA patients. To address the effects of reduced levels of frataxin in a mammalian system, we are also developing mutant mouse lines in which the frataxin gene carries a targeted insertion of a hygromycin B-poly(A) cassette in the first intron, which is predicted to produce heterozygous and homozygous mice with graded expression of frataxin. A genomic clone of ~18 kb containing exons 1-3 of the mouse frataxin gene has been isolated and restriction sites for construction of the targeting vector have been identified. This work is supported by the Muscular Dystrophy Association and the National Institute on Aging. P.C. is supported by the P. and L. Mariani Foundation, Milan, Italy.

Program Nr: 1613 from the 1999 ASHG Annual Meeting

Novel prion protein sequence variant associated with familial encephalopathy. *L. Cervenakova[1], C. Buetefisch[1], H-S. Lee[1], I. Taller[1], G. Stone[1], C.J. Gibbs, Jr.[1], P. Brown[1], M. Hallett[1], P. Gambetti[2], L.G. Goldfarb[1]*. 1) National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD 20892; 2) Case Western Reserve University, Cleveland, OH 44106.

Hereditary spongiform encephalopathies (hSE) are associated with mutations in the PRNP (prion protein) gene on chromosome 20p12-pter. We report a four-generation family, in which seven patients developed limb and truncal ataxia, dysarthria, myoclonic jerks and cognitive decline. The pattern of inheritance was autosomal dominant. The age of onset in the 30s, 40s or 50s, prolonged disease duration, cerebellar atrophy on imaging and the presence of synchronic periodic discharges on EEG suggested a familial encephalopathy resembling Gerstmann-Straussler-Scheinker disease. PrP deposits with laminar distribution in the cerebral cortex were identified in a biopsy sample obtained from one of the patients. A previously unknown A-to-G transition in codon 187 changing the codon sequence from CAC to CGC and resulting in a predicted histidine to arginine (H187R) substitution was detected in affected, but not in unaffected family members or 49 unrelated control individuals, suggesting a pathogenic role for this mutation. This novel H187R mutation is located within the third alpha-helical segment of the prion protein, in which other pathogenic mutations causing hSE have been identified.

Program Nr: 1614 from the 1999 ASHG Annual Meeting

Mutation analysis of prion gene in Chinese schizophrenic patients from Taiwan. *C.H. Chen[1,2], M.T. Tsai[2]*. 1) Department of Psychiatry, Tzu-Chi General Hosp, Hualien City, Taiwan; 2) Institute of Human Genetics, Tzu-CHi Medical College, Hualien City, Taiwan.

Prion disease may present as inherited, infectious and sporadic neurodegenerative disorders,resulting from aberrant prion protein conformation. These disorders, characterized by spongiform encephalopathy in the cerebral cortex, include Creutzfeldt-Jakob disease (CJD), kuru, Gerstmann-Straussler disease(GSD) and familial fatal insomnia (FFI). Recently, a new variant of CJD (vCJD) was observed. The psychiatric manifestations in the initial presentation in these cases raised the possibility that prion gene mutation may be associated with serious psychiatric disorders. In fact, Samaia and colleagues recently reported an asparagine to serine mutation at codon 171 (N171S) of prion gene in a family with serious psychiatric disorder (Nature 1997;390:214). Prompted by this finding, we first systematically sequenced the protein coding sequences of prion gene in 60 Chinese schizophrenic patients with family history from Taiwan. We identified two polymorphisms of prion gene in this cohort. However, no other mutation including N171S was identified. These two polymorphisms include methionine/valine at codon 129 (M129V) (allele frequencies: Met 96%, Val 4%) and glutamic acid/lysine at codon 219 (E219K) (allele frequencies: Glu 96%, Lys 4%). The M129V was found in both Caucasians and Orientals, while E219K was only found in Japan, not in Caucasians. Our data add further support that E219K may only occur in Orientals. Furthermore, genotype distributions and allele frequencies of these two polymorphisms were determined in 235 schizophrenic patients and 100 non-psychiatric controls. No significant difference was found between two groups. We further compared the estimated haplotype frequencies of these two polymorphisms between patients and controls. Similarly, no significant difference was identified. In conclusion, our study demonstrated that prion gene mutation may not play a major role in the pathogenesis of schizophrenia in Chinese population.
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Program Nr: 1615 from the 1999 ASHG Annual Meeting

The Familial Mediterranean Fever protein interacts and colocalizes with a putative Golgi transporter. *X. Chen, Y. Bykhovskaya, M. Hamon, Z. Bercovitz, O. Spirina, N. Fischel-Ghodsian.* Ahmanson Department of Pediatrics, Steven Spielberg Pediatric Research Center, Medical Genetics Birth Defects Center, Cedars-Sinai Medical Center and UCLA School of Medicine, Los Angeles, CA.

The biological function of pyrin, the protein mutated in Familial Mediterranean fever (FMF), has not been elucidated. Based on sequence homology, a transcription factor activity was proposed for this neutrophil specific protein. In a yeast two hybrid assay, neither transcription activation activity nor any self interaction was detected for pyrin. Screening of an expression cDNA library of peripheral blood leukocytes using as bait the carboxyl portion of pyrin (amino acids 557-781) which contains most of the FMF mutations, led to the identification of P/M-IP1 (pyrin/marenostrin interacting protein 1). A splice variant of P/M-IP1, GTC-90, had previously been described as a component of the 13 S hetero-oligomeric protein complex that stimulates in vitro Golgi transport. We have now shown that P/M-IP1 colocalizes with pyrin in the perinuclear cytoplasm of Cos-7 cells, and that the interaction between these two proteins is impaired by FMF causing mutations in pyrin. These data suggest that, at some stage of its functional pathway, pyrin resides in the cytoplasm and might be involved in, or impacted by, cellular protein sorting by the Golgi apparatus. The data also imply that P/M-IP1 may be involved in the abnormal inflammatory response which occurs in patients with FMF.

Program Nr: 1616 from the 1999 ASHG Annual Meeting

Association between changes with time in the level of aberrantly spliced CFTR mRNA and CF lung disease severity. *O. Chiba-Falek[1], E. Kerem[2], M. Nissim-Raffinia[1], A. Tal[3], M. Aviram[3], A. Augarten[4], B. Kerem[1].* 1) Dept Genetics, Hebrew Univ, Jerusalem, Israel; 2) Dept Pediatrics and CF Center Shaare Zedek Medical Center, Hebrew Univ Medical School, Jerusalem, Israel; 3) Dept Pediatrics and CF Clinic, Soroka Medical Center, Ben Gurion Univ, Beer Sheva, Israel; 4) Dept Pediatrics and CF Clinic, Chaim Sheba Medical Center, Tel Hashomer, Israel.

The disease severity of cystic fibrosis patients carrying splicing mutations correlates with the level of aberrantly spliced CFTR transcripts. The aim of this research was to investigate if there are changes with time in the level of aberrantly spliced transcripts and if such changes might be associated with changes in disease severity. We analyzed the levels of aberrantly spliced RNA transcribed from the splicing mutation 3849+10kb C®T, in nasal epithelial cells from five CF patients at two time points, 1-4 years apart. Semi-quantitative non-differential RT-PCR of the samples, revealed considerable variability in the levels of the aberrantly spliced mRNA among the patients (0-28%). These levels correlated with the lung disease severity of the patients ($r=0.94$, $p=0.015$). Comparison of the levels of aberrantly spliced CFTR mRNA transcribed from the 3849+10kb C®T alleles at the two different time points, showed that changes in lung disease severity were associated with changes in the level of aberrantly spliced CFTR mRNA. Higher levels of aberrantly spliced CFTR mRNA in the second analysis were associated with declines in pulmonary functions. Lower levels of aberrantly spliced CFTR mRNA at the second time point were associated with lung function improvement. Normal lung function at both time points was associated with the presence of normal CFTR transcripts only. Thus, the regulation of alternative splice site selection may be an important mechanism underlying changes with time in CF disease severity in patients carrying splicing mutations. The same mechanism might underlie changes overtime in disease severity observed in other human genetic diseases caused by splicing mutations.

Program Nr: 1617 from the 1999 ASHG Annual Meeting

Activating Mutations in Fgfr3 are Responsible for Decreased Receptor Degradation. *Y. Cho[1,2], W.A. Horton[2]*. 1) Dept of Mol & Medical Genetics, Oregon Health Sciences Univ., Portland, OR; 2) Research Dept, Shriners Hospital for Children, Portland, OR.

Fibroblast growth factor receptor 3 (Fgfr3) is an important regulator of endochondral bone growth and mutations in Fgfr3 are responsible for the chondrodysplasia class of dwarfing conditions in humans. The pathogenesis of Fgfr3 mutations in inhibiting bone growth has been attributed in part to constitutive activation of the receptor, which in turn activates other signaling pathways such as STAT1/p21, the MAP kinases, and PLC-gamma. Here we propose an additional means by which mutations in Fgfr3 enhance signaling. We show that activating mutations in Fgfr3 lead to an accumulation of FGFR3 protein within the cell, in comparison to cells expressing wild type receptors. The accumulation of mutant proteins occurs despite similar RNA levels, suggesting the activated mutant Fgfr3 is not being degraded properly. This may be due to a problem with the processing of the receptor through the proteasome machinery or perhaps an accumulation of the mutant receptor in the ER/Golgi apparatus. To determine the role of the proteasome pathway in the degradation of FGFR3, we have studied the effects of proteasome inhibitors (lactacystin) and enhacers (geldanamycin, genestein, herbimycin) on cells transfected with mutant or wt Fgfr3. As well, we have generated a fusion protein in which the C-terminus of FGFR3 has been tagged with EGFP (Fgfr3-EGFP). We show the EGFP fusion does not affect the signaling properties of FGFR3 nor does FGFR3 affect the fluorescent properties of the EGFP. Using confocal microscopy, we have visualized an accumulation of FGFR3-EGFP in certain subcellular compartments.

Program Nr: 1618 from the 1999 ASHG Annual Meeting

Long QT syndrome: Genotype-phenotype relationship and relation to Sudden infant death syndrome (SIDS). *M. Christiansen[1], L.A. Larsen[1], I. Fosdal[2], I.H. Svendsen[3], P.S. Andersen[1], J.K. Kanters[4], J. Vuust[1], G. Wettrell[5].* 1) Dept Clinical Biochemistry, Statens Serum Inst, Copenhagen, Denmark; 2) Dept Pediatrics, Visby Hospital, Visby Gotland, Sweden; 3) Dept Medicine B, State University Hospital, Rigshopitalet, Copenhagen, Denmark; 4) Dept of Medical Physiology, University of Copenhagen, Denmark; 5) Dept of pediatric Cardiology, Lund University Hospital, Lund, Sweden.

Long QT syndrome (LQTS) is a genetic disease of the heart characterized by a prolonged QT interval on the ECG and a propensity for tachyarrhythmias, causing syncopes and sudden cardiac death. Recently, mutations in the genes KVLQT1, HERG, SCN5A, KCNE1, and MIRP1, coding for subunits of cardiac ion channels have been found to cause LQTS. In 12 Scandinavian families with LQTS we found seven different missense mutations and one trinucleotide duplication in HERG. In SCN5A we found one splice-site mutation, and in KVLQT1 we found one splice-site, one nonsense, and 3 missense mutations. No mutations were found in neither KCNE1 nor MIRP1. In 10 families the clinical picture was classical Romano-Ward syndrome with variable penetrance, but in two families LQTS exhibited recessive inheritance. At present there is no evidence of a founder effect, and the mutations causing LQTS seem to be private. Mutations in HERG seemed to have a worse prognosis than mutations in other genes. In two families with SIDS we found mutations in the PAS domain of the HERG K-channel. A pilot-study in 120 patients dying from SIDS revealed no pore-associated mutations in neither KVLQT1 nor HERG, or mutations in the c-terminus of KVLQT1, or in KCNE1. We suggest that the documented association between a pro-longed QT-interval on the ECG in newborns and the risk of SIDS could be caused by mutations in regulatory domains of the HERG ion channel. .

Program Nr: 1619 from the 1999 ASHG Annual Meeting

Graduated strategy for the molecular diagnosis of craniosynostosis syndromes. *K.M. Chun[1], A. Teebi[2], S. Kennedy[2], C.R. Forrest[3], P.N. Ray[1]*. 1) Depts. of Genetics and Pediatric Lab Medicine; 2) Dept. of Pediatrics; 3) Dept. of Surgery, Hospital for Sick Children, Toronto, Canada.

Craniosynostosis is the premature fusion of calvarial bones leading to an abnormal head shape. The craniosynostosis syndromes are clinically heterogeneous with overlapping features, which make an accurate diagnosis very difficult. Although accurate diagnosis, namely the clarification of a genetic lesion, does not have a direct impact on patient management in a lot of these cases, there is a significant benefit with accurate prenatal diagnosis. Genetic counsellors are able to offer better risk estimates of recurrences to non-manifesting carriers and their extended family members and for affected patients of reproductive age. Recent advances in gene discovery have shown that these craniosynostosis syndromes, with the exception of Apert syndrome, are genetically heterogeneous. Apert patients have a missense mutation in exon IIIa of the fibroblast growth factor receptor 2 (*FGFR2*) gene that affects codons 252 and 253. Crouzon and Pfeiffer patients have been shown to carry a variety of mutations in and around exons IIIa and IIIc of *FGFR2*, and mutations in *TWIST* have been shown to be the underlying defect in a number of Saethre-Chotzen patients. The recently defined non-syndromic craniosynostosis syndrome generally is due to a recurrent P250R mutation in *FGFR3*. However, this mutation has also been found in patients who were initially clinically diagnosed with Crouzon, Pfeiffer or Saethre-Chotzen syndrome. A recurrent mutation in *FGFR1* accounts for the familial cases of Pfeiffer syndrome. We surveyed 91 craniosynostosis patients at the molecular level and have found mutations in approximately half of them. Of note are two novel Pfeiffer mutations, both of which affect the 5' splice site of exon IIIc of *FGFR2*. They are 952-1G®A and 952-3del10insACC. In studying these 91 patients, a diagnostic strategy for craniosynostosis testing became apparent, where sequential analysis of recurrent mutations is followed by selective sequencing. This algorithm makes testing of craniosynostosis syndromes more efficient and cost-effective for the diagnostic laboratory.

Program Nr: 1620 from the 1999 ASHG Annual Meeting

Restricted distribution of loss-of-function mutations within the LMX1B genes of nail patella syndrome patients.
M.V. Clough, J.D. Hamlington, I. McIntosh. Institute of Genetic Medicine, Johns Hopkins Univ., Baltimore, MD.

Nail patella syndrome (NPS) is a pleiotropic condition characterized by dysplasia of the nails, hypoplasia of the patellae, elbow dysplasia and progressive kidney disease. The syndrome is inherited in an autosomal dominant manner and has been shown to result from mutations in the LIM-homeodomain encoding LMX1B gene. The LMX1B transcription factor plays a role in defining the development of dorsal specific structures during limb development; its role in other organs is unclear. The identification of 29 mutations published previously suggests that NPS is the result of haploinsufficiency for LMX1B (AJHG 63:1651, 1998). A further 39 mutations have been identified comprising 14 missense, 9 nonsense, 8 small insertions/deletions, 4 putative splice mutations and 4 larger deletions. Each of 22 missense mutations identified to date involves substitution of a conserved residue in either the LIM or homeodomains. All of 33 nonsense and frameshift mutations which would be predicted to prevent synthesis of full length polypeptides are also concentrated in and around these functional domains. No NPS mutations have been observed within the carboxy-terminal third of the coding sequence suggesting that mutations in this region are not inactivating or, at least, do not prevent LMX1B acting to dorsalize the developing limb. Any other phenotype resulting from mutation of this domain remains to be identified. The function of the C-terminal glutamine-rich region is under investigation. These findings support the hypothesis that NPS results from a 50% reduction in LMX1B function via a reduction in synthesis, disruption of secondary structure or failure to bind DNA.

Program Nr: 1621 from the 1999 ASHG Annual Meeting

***Jagged1* Mutations in Alagille Syndrome Patients and Their Families.** *R.P. Colliton[1,2], A. Genin[1,2], L. Bason[1,2], D.A. Piccoli[1,3], I.D. Krantz[1,2], N.B. Spinner[1,2].* 1) The Children's Hospital of Philadelphia, Phila, PA; 2) Div. Human Genetics; 3) Div. of Gastroenterology.

Alagille syndrome(AGS)is a dominant multisystem developmental disorder previously shown to result from mutations in Jagged1(JAG1),a ligand in the Notch signaling pathway.Clinically,AGS is characterized by bile duct paucity in combination with heart defects,ocular anomalies,vertebral anomalies and characteristic facial features.As part of our interest in understanding the full spectrum of JAG1 mutations associated with this disorder,and the clinical features associated with mutations in JAG1,we have studied a large cohort of patients with Alagille syndrome,and their family members,to identify mutations in JAG1.This study reports our current experience.To date,we have completely screened 127 probands for mutations in JAG1,by SSCP and identified 75 mutations(59%).Most of the mutations(72%)are protein truncating with 8%caused by splicing mutations,12%missense mutations and 4%total gene deletions.The mutations occur throughout the coding sequence of JAG1,within the evolutionarily conserved protein motifs. Very few of the mutations were seen in more than one unrelated patient,the exceptions being 2 small deletions in exons 16(2 pts.)and 17(3 pts.)and a small insertion in exon 9(3 pts.)all of which occurred where there were repeated sequences within the gene.The 9 missense mutations occurred at 6 different amino acids within the extracellular portion of JAG1.Parental samples were available for screening in 40 cases, and in 16 (40%)the mutations were inherited (8 maternal and 8 paternal).In the families in which there was an inherited mutation,a total of 38 JAG1 mutation carriers were identified. Clinical features in these individuals ranged from very mild to full blown AGS,with most individuals not meeting clinical criteria for the disorder.To identify the cause of disease in the 40%of AGS patients screened,in whom no mutation could be found,we are currently screening JAG1 cDNA using conformational strand gel electrophoresis(CSGE).This an alternative electrophoretic technique which has proven to be highly sensitive in studies of other genes.Results of these studies will be reported.

Program Nr: 1622 from the 1999 ASHG Annual Meeting

A novel mutation (Phe383Cys) in a patient with Achondroplasia carrying a classic Gly380Arg mutation. *D. Concolino[1], M. Baldi[2], M. Baffico[2], S. De Seta[1], T. Viscomi[1], G. Camera[3], P. Strisciuglio[1]*. 1) Dept. of Pediatrics, University of Catanzaro, Italy, MD; 2) Lab. of Human Genetics, Hospital "Galliera" of Genoa, Italy, PhD; 3) Surgical Center of Hand, Savona, Italy, MD.

Achondroplasia, the commonest form of non lethal osteochondrodysplasias, is inherited as autosomal dominant trait and is derived from a defective endochondral ossification which leads to a delayed growth of long bones with disharmonic dwarfism. This condition is usually sporadic and is associated with advanced paternal age. The achondroplasia gene, mapped to chromosome 4p16.3, encodes fibroblast growth factor receptor 3. Recently, several mutation have been identified, 95% of which result in a glycine to arginine substitution at codon 380 (Gly380Arg). Less common mutations are Gly375Cys and Gly346Glu. We present a case of achondroplasia with the classic Gly380Arg mutation on the paternal allele and a novel mutation Phe383Cys on the maternal allele. At admission in our hospital, the 3 days old patient showed rhizomelic shortness of limbs, large cutaneous folds of arms, craniofacial deformities such as macrocephaly, prominent forehead, flattened nasal root. Skeletal X rays confirmed the diagnosis of achondroplasia. Molecular analysis showed a point mutation T®G at the nucleotide 1148 (Phe383Cys) in additin to the classic mutation Gly380Arg. The same mutation Phe383Cys was detected in the asymptomatic mother. The point mutation T®C at the nucleotide 1350 (Phe384Leu) is found in 0.04% of achondroplasic patients, and it is considered a polymorfism, because its presence in normal population. This mutation leads to substitution from phenylalanine to cysteine, either neutral and hydrofobic aminoacids, that may explain absence of disease in the carrier. On the other hand, the mutation Phe383Cys, never reported previously, results in the substitution of a neutral hydrofobic aminoacid in a neutral polar one, so that may produce an alterated protein. Further reports are necessary to understand the consequences of this mutation.

Program Nr: 1623 from the 1999 ASHG Annual Meeting

ULTRASTRUCTURE OF CORNEAL EPITHELIUM FROM A PATIENT WITH MEESMANN'S CORNEAL DYSTROPHY AND NOVEL MUTATIONS IN KERATIN 12. *L.D. Corden[1], C.M. Coleman[1], A.D. Irvine[2], O. Swensson[3], B. Swensson[4], R. Rochels[4], G.C.M. Black[5], W.H.I. McLean[1]*. 1) Epithelial Genetics Group, Dundee, UK; 2) Dermatology, Belfast, UK; 3) Dermatology, Kiel, Germany; 4) Ophthalmology, Kiel, Germany; 5) Clinical Genetics, Manchester, UK.

Recently, we identified the first mutations in corneal keratins K3 and K12 in kindreds with Meesmann's corneal dystrophy (MCD). Here, we report 6 further K12 mutations and ultrastructural analysis of MCD cornea. Mutation 428G>C was identified in a German family, predicting amino acid change R135T. This mutation represents an ancestral German mutation. In another German kindred, we detected novel mutation 413A>C, predicting amino acid substitution Q130P. A novel mutation was detected in an American kindred, 410T>C, which predicts the amino acid substitution M129T. The other three novel mutations found were: 451G>T (V143L) in a kindred from Saudi Arabia; 423T>G (N135K) in an English kindred and 1300A>G (I426V) in a second American kindred. The mutations were found to co-segregate with MCD in the families and were excluded from 100 normal chromosomes by restriction enzyme analysis. All 6 mutations occur in the helix boundary motifs which are highly conserved between intermediate filament proteins. Mutations in these regions in other keratins have previously been shown to severely compromise cytoskeletal function, leading to phenotypes characterised by epithelial fragility. A total of thirteen mutations have now been reported in the K12 gene. In addition to the genetic analysis, ultrastructural examination was performed on corneal tissue from one MCD patient carrying the N135K mutation. This revealed perinuclear vacuolation, basement membrane abnormalities and cellular degeneration. In addition, thickened, shortened and clumped filamentous structures were seen, highly analogous to the keratin filament aggregates in the skin disease epidermolysis bullosa simplex, caused by mutations in the basal cell keratins K5 and K14. Thus, these abnormal subcellular structures were recognised for the first time as keratin filament aggregates, providing further evidence for the pathologic relevance of keratin mutations in MCD.

Program Nr: 1624 from the 1999 ASHG Annual Meeting

Characterization of candidate genes for Microphthalmia with Linear Skin Defects (MLS) through generation of targeted deletions in the mouse. *T.A. Cormier[1], I.B. Van den Veyver[1], S.K. Prakash[1], A. McCall[1], B. Wu[1], H.Y. Zoghbi[1,2]*. 1) Baylor College of Medicine, Houston, TX; 2) Howard Hughes Medical Institute, Houston, TX.

MLS is a disorder caused by a dominant, male lethal deletion of Xpter to Xp22.3. Heterozygous females have microphthalmia, skin lesions on the head and neck, agenesis of the corpus callosum and mental retardation. We propose that the loss of function of one or more previously isolated genes (*ARHGAP6*, *HCCS* and *MID1*) from the 450 Kb candidate region causes MLS. Because MLS is caused by deletions, mutation analysis of candidate genes in patients is not possible. Therefore, to understand the contribution of each gene, we set out to generate inactivating mutations in *arhgap6*, *hccs* and *mid1*, and to engineer targeted deletions of the homologous region in the mouse. We targeted each gene in ES cells with selectable markers and one *lox*P site (using either replacement or insertion vectors). The markers and *lox*P sites were oriented to generate specific deletions in the region using Cre recombinase.

We replaced *61b3r* (the homologue of a human 5' UTR exon of *MID1* at the telomeric boundary of the region) with exons 3-9 of HPRT. *61b3r* was also replaced in an ES cell line previously targeted at the *arhgap6* locus (at the centromeric boundary of the region). Both $61b3r^{-}$ and $61b3r^{+/-}$/$arhgap6^{+/-}$ animals are apparently unaffected by the replacements. $61b3r^{+/-}$/$arhgap6^{+/-}$ females are being mated to males transgenic for Cre recombinase under an EIIa promoter which then drives expression in the zygote. The offspring of this mating are expected to be models for human MLS.

A vector with exons 3-9 of HPRT was inserted into exon 1 of the *mid1* gene. We have generated a $mid1^{ins/+}$/$arhgap6^{+/-}$ mouse which will be used to generate a deletion larger than *61b3r*/*arhgap6*. Characterization of these mice will establish the role of *MID1* in the MLS phenotype.

Program Nr: 1625 from the 1999 ASHG Annual Meeting

Friedreich ataxia mouse models. *M. Cossee, H. Puccio, H. Koutnikova, V. Campuzano, K. Fischbeck, J.L. Mandel, M. Koenig.* Inst Genetics, Illkirch Strasbourg, Illkirch, France.

Friedreich ataxia is caused by the loss of function of frataxin, a mitochondrial protein conserved through evolution. Yeast knock-out models, histological and biochemical data from heart biopsies or autopsies from patients suggest that frataxin may play a role in mitochondrial iron transport or in iron-sulfur proteins assembly. Affected human tissues are rarely available to test this hypothesis. We generated a mouse model by inactivation of the frataxin gene homologue. The gene disruption was obtained by deletion of exon 4, which causes a frame-shift. The genotype of surviving offsprings from heterozygote intercrosses was either wild-type or heterozygous; no mouse was homozygous for the deleted allele. We are analysing embryos to investigate the stage of embryonic lethality and the mechanism of degeneration, with a particular emphasis on iron accumulation. To circumvent embryonic lethality, we are generating a conditional knock-out model, based on the Cre-lox system in which exon 4 will be deleted under temporal and tissue specific control, and a knock-in mouse model by insertion of the H180R missense mutation found in a typical Friedreich ataxia patient (H183R). These models will allow to investigate the mechanism of the disease and to test anti-oxidant therapies.

Program Nr: 1626 from the 1999 ASHG Annual Meeting

Severe mutations in the *ABCR* gene are associated with a clinical subtype of autosomal recessive retinitis pigmentosa. *F. Cremers[1], A. Maugeri[1], J. Klevering[2], S.L. Go[2], A. Wagner[3], H. Brunner[1], C. Hoyng[2].* 1) Dept Human Genetics, Univ Hosp Nijmegen, Nijmegen, Netherlands; 2) Dept Ophthalmology, Univ Hosp Nijmegen, Nijmegen, Netherlands; 3) Dept Clinical Genetics, Erasmus Univ, Rotterdam, Netherlands.

Mutations in the retina specific ATP-binding cassette transporter (*ABCR*) gene are associated with Stargardt disease (STGD) and an as yet unknown fraction of autosomal recessive (ar) retinitis pigmentosa (RP) and cone-rod dystrophy (CRD). In addition, heterozygous ABCR mutations increase the risk for age-related macular degeneration (AMD). Our studies have shown that *ABCR* mutations can be grouped in different classes of severity and that the severity of the retinal disease is inversely correlated with the remaining activity of the ABCR protein. In patients with the most severe phenotype, RP, both *ABCR* genes carry null mutations.

Clinically, RP patients with *ABCR* mutations can be distinguished from other RP patients because they show a more severe visual loss due to an early macular degeneration. Based on our model we estimated that *ABCR* mutations might account for a sizeable fraction (10-15%) of arRP. In this study, we have selected a group of 15 sporadic and two novel familial cases with RP which show the typical early macular involvement. Upon SSCP screening of approximately one-third of the *ABCR* gene, we identified *ABCR* mutations in at least 3 patients with RP. In a family in which patients with RP and STGD are second counsins and the father of the RP patient shows AMD, a homozygous splice site mutation (768G®T) was found in the RP patient, whereas the STGD patient carries the same splice site mutation and a mild 2588G®C founder mutation. Another sporadic RP patient was also shown to be homozygous for the 768G®T mutation. A sporadic patient with RP shows a deletion of exons 20-22 on one allele and a splice site mutation (IVS28-2A®G) in the other allele. In conclusion, this study confirms a relatively frequent involvement of the *ABCR* gene in arRP, in particular in patients with an early macular involvement.

Program Nr: 1627 from the 1999 ASHG Annual Meeting

An approach for screening of Hereditary Hyperferritinemia Cataract Syndrome (HHCS) by a new DG-DGGE method for rapid mutational scanning in ferritin L-chain IRE. *L. Cremonesi[1], A. Fumagalli[1], N. Soriani[1], M. Ferrari[1], E. Tinazzi[2], M. Camparini[3], R. Aldigeri[3], D. Girelli[2], P. Arosio[4], S. Levi[5].* 1) Unit of Genetics and Molecular Diagnostics, IRCCS, H. San Raffaele, Milan, Italy; 2) Department of Clinical and Experimental Medicine, University of Verona, Italy; 3) Institute of Ophthalmology, University of Parma, Italy; 4) Chair of Chemistry, University of Brescia, Spedali Civili, Brescia, Italy; 5) Protein Engineering, IRCCS, H. San Raffaele, Milan, Italy.

HHCS is an autosomal dominant disorder characterized by elevated serum ferritin levels, unrelated to iron status, and early onset bilateral cataract. The disease is caused by heterogenous mutations on the iron responsive element (IRE) sequence in the noncoding region of L-ferritin. The IRE mutations so far reported have been identified by DNA sequencing. We developed double-gradient denaturing gradient gel electrophoresis (DG-DGGE) conditions to easily identify sequence alterations. By this method we analyzed seven known mutations (G41C, A40G, C39T, G32A, T22G/C18T, D10-38, G51C), representing the majority of the mutations so far described. All mutations displayed an altered electrophoretic pattern. Thus, the optimized DG-DGGE technique appears a powerful and rapid method for scanning L-ferritin IRE and study the prevalence of the mutations in hyperferritinemias and juvenile cataracts, and to evaluate the presence of asymptomatic polymorphisms in the sequence region. In collaboration with an Ophthalmologycal center, where more than 2,000 cataract operations (mostly age-related) are performed per year, we started a pilot study to evaluate the frequency of HHCS in cataracts. We selected 50 cases including "unexplained" juvenile cataract (mean age 33.4), senile cataracts and age-matched controls. All samples were negative for IRE mutations. The work has been extended to a selected population of 20 cases with juvenile cataract and hyperferritinemia (more than 400 mg/L). We are planning an approach to screen for HHCS mutations in a large population of blood donors and subjects with hyperferritinemia (more than 500 mg/L), with cataract. (Partially supported by a grant of Telethon-Italy).

Program Nr: 1628 from the 1999 ASHG Annual Meeting

Correlation of sarcospan and sarcoglycan expression in patients with muscular dystrophies. *R.H. Crosbie[1], H. Collin[2], S. Moore[3], F.M.S. Tomé[2], M. Fardeau[2], K.P. Campbell[1].* 1) Howard Hughes Medical Institute, Dept. of Physiology & Biophysics, Dept. of Neurology, University of Iowa College of Medicine, Iowa City, IA; 2) INSERM 523, Institut de Myologie, Hôpital de la Salpêtrière, Paris; 3) Dept. of Pathology, University of Iowa College of Medicine, Iowa City, IA.

We recently identified a unique member of the dystrophin-associated glycoprotein complex, which we have named sarcospan based on its multiple sarcolemma-spanning domains (Crosbie et al.,1997). The gene encoding sarcospan is localized to human chromosome 12p11.2 and to date, no mutations in this gene have been found to cause muscle-related disorders. In order to assess the potential role of sarcospan in disease pathogenesis and to further probe the molecular associations of sarcospan with other proteins of the dystrophin-glycoprotein complex, we examined sarcospan expression in muscle from a panel of sarcoglycan-deficient limb-girdle muscular dystrophy patients. Primary mutations in a, b,g, or d-sarcoglycan disrupt the structural organization of the sarcoglycan complex and consequently result in LGMD-2D, 2E, 2C and 2F, respectively. In summary, we demonstrate that sarcospan expression is correlated with the level of sarcoglycan expression in muscle from LGMD patients. Sarcospan is dramatically reduced in all sarcoglycan-deficient patients examined. We show that sarcospan is tightly associated with the sarcoglycan complex and that membrane targeting and localization of sarcospan is dependent on the sarcoglycan complex. In order to determine if primary mutations in sarcospan are associated with human disease, we have developed a PCR-based screening assay and have identified intragenic polymorphic markers that are important for linkage analysis. Our data provide important implications about sarcospan's involvement in as yet uncharacterized forms of muscular dystrophy.

Program Nr: 1629 from the 1999 ASHG Annual Meeting

Aberrant splicing in the presenilin-1 intron 4 mutation causes presenile Alzheimers disease by increased ab**42 secretion.** *M. Cruts[1], C. De Jonghe[1], E.A. Rogaeva[2], C. Tysoe[3], A. Singleton[3], H. Vanderstichele[4], W. Meschino[2], B. Dermaut[1], I. Vanderhoeven[1], H. Backhovens[1], E. Vanmechelen[4], C.M. Morris[3], J. Hardy[5], D.C. Rubinsztein[3], P.H. St.George-Hyslop[2], C. Van Broeckhoven[1].* 1) Flanders Interuniversity Institute for Biotechnology (VIB), Born-Bunge Foundation (BBS), University of Antwerp (UIA), Department of Biochemistry, Antwerpen, Belgium; 2) Centre for Research in Neurodegenerative Disease, University of Toronto and North York General Hospital, Ontario, Canada; 3) Cambridge Institute for Medical research, Wellcome/MRC Building, Addenbrookes Hospital, Cambridge and MRC Neurochemical Pathology Unit, Newcastle General Hospital, Newcastle upon Tyne, UK; 4) Innogenetics Inc., Zwijnaarde, Belgium; 5) Neurogenetics Laboratory, Mayo Clinic Jacksonville, Florida, USA.

We previously described a splice donor site mutation in intron 4 of presenilin-1 (PSEN1) in 2 autopsy-confirmed patients with early-onset Alzheimer's disease (AD). Since then, we identified 4 additional AD cases. The mutation segregates in an autosomal dominant manner and genotype analysis of markers located near PSEN1 indicated that all cases have one common ancestor. The mutation involves a deletion of the first nucleotide of intron 4 destroying the spice donor consensus sequence and produces 3 different transcripts due to aberrant splicing: 2 deletion transcripts (D4 and $D4_{cryptic}$) and 1 insertion transcript (ins_{TAC}). The deletion transcripts result in the formation of C-truncated PSEN1 proteins while the insertion transcript produces full-length PSEN1 with one extra amino acid (Thr) inserted between codons 113 and 114 (PSEN1 T113-114ins). *In vivo*, the truncated proteins were not detectable in brain homogenates or lymphoblast lysates of mutation carriers. *In vitro*, HEK-293 cells overexpressing D4, $D4_{cryptic}$ or ins_{TAC} PSEN1 cDNAs showed increased Ab42 secretion (~3.4 times) only for the insertion cDNA construct. Increased Ab42 production was also observed in brain homogenates. Our data indicate that in case of the intron 4 mutation, the AD pathophysiology results from the presence of the PSEN1 T113-114ins protein comparable to cases carrying PSEN1 missense mutations.

Program Nr: 1630 from the 1999 ASHG Annual Meeting

The TaqI(B) Polymorphism in the CETP Gene is Associated with Variation in High Density Lipoprotein. *L.A. Cupples[1], J.M. Ordovas[2], P.W.F. Wilson[3,4], E.J. Schaefer[2].* 1) Dept Epid/Biostatistics, Boston Univ Sch Public Health, Boston, MA; 2) Tufts University, Boston, MA; 3) Boston Univ Sch Medicine, Boston, MA; 4) Framingham Heart Study, NIH/NHLBI, Framingham, MA.

High levels of high density lipoprotein cholesterol (HDL-C) are known to be protective against the development of coronary heart disease while low levels are known to be a risk factor. Cholesteryl ester transfer protein (CETP) facilitates the exchange of triglycerides and cholesteryl ester between lipoprotein particles. The CETP gene has been localized to 16q21 and encompasses 16 exons. CETP deficiency is associated with hyperalphalipoproteinemia, primarily due to an increase of cholesteryl ester-enriched large-sized HDL. The TaqI(B) polymorphism (RFLP) in the CETP gene was typed in 2916 subjects from the Framingham Offspring Study with the goal of examining its relationship to a variety of lipid measures, including total cholesterol (TC), HDL-C, LDL-C and triglycerides. The B2 allele at this site has been previously associated with increased HDL-C levels. To evaluate its relationship to a variety of lipid measures, we performed measured genotype analyses with this polymorphism, classifying subjects as B1B1, B1B2 or B2B2. These analyses were performed using the variance component approach implemented in SOLAR adjusting for gender, age, body mass index, smoking, alcohol intake, use of beta blockers, apolipoprotein E, menopausal status and estrogen replacements in women. The results of this analysis indicate an increasing gradient in HDL-C levels with an increase in the number of B2 alleles ($p < 0.0001$). A similar result is found for HDL-C subfractions, HDL2 and HDL3. The TC/HDL-C ratio shows a corresponding decreasing gradient with an increase in the number of B2 alleles. A genome scan of 1535 subjects in 236 Framingham families revealed no lod scores above 0.5 in this region on chromosome 16. Since the estimated proportion of variation in HDL-C in our measured genotype analysis due to the TaqI(B) polymorphism is approximately 1 percent, we conclude that identification of genes need to be tackled with both candidate gene and linkage approaches.

Program Nr: 1631 from the 1999 ASHG Annual Meeting

Characterization of SMN hybrid genes in Spanish SMA patients: evidence of *de novo* and compound heterozygous cases. *I. Cusco[1], M.J. Barcelo[1], E. Bussaglia[2], M. Baiget[1], E. Tizzano[1]*. 1) Genetics, Hospital Sant Pau, Barcelona, Spain; 2) Pathology,Hospital Sant Pau, Barcelona, Spain.

Spinal muscular atrophy (SMA) is classified in type I (severe form), type II (intermediate form) and type III (mild/moderate form) and is caused by mutations in the Survival Motor Neuron telomeric (SMNt) gene. A centromeric functional copy of this gene (SMNc) is deleted in 10% of the control Spanish population. Approximately in 85% of the Spanish SMA patients, a homozygous deletion of exon 7 and 8 of the SMNt gene is detected regardless of the phenotype. In addition 5% of the cases have only a deletion of exon 7. We analyzed 14 unrelated cases with this mutation to characterize centromeric-telomeric hybrid genes. We have studied a possible phenotype-genotype correlation comparing the different structures of the hybrid alleles, the presence or absence of NAIP and the origin of the mutation in their parents. Twelve patients showed a CCT structure (Cent intron 6-Cent exon 7- Tel exon 8) characteristic of a gene conversion event. However one case showed concomitantly a homozygous deletion of the centromeric exon 8, indicating that an intrachromosomal deletion was involved. Two cases were type I, 7 type II and 3 type III. Nine patients retained at least one copy of NAIP exon 5 (being present in the 3 type III cases). All but one CCT case were inherited. The exemption was probably a *de novo* case although germinal mosaicism could not be excluded. Another type II patient showed the hybrid structure TCT (Tel intron 6-Cent exon 7- Tel exon 8) with deletion of NAIP and the remaining case was a very mild affected woman with a CCT structure in one chromosome and a TCT in the other with presence of NAIP. We conclude that: 1) in all type III cases at least one copy of NAIP 5 was detected, while this gene was absent only in types I and II hybrid chromosomes; 2) no association with the paternal or maternal origin of the mutation was found 3) *de novo* hybrid genes may account for a small but still important proportion of the cases; 4) the presence of two chromosomes with gene conversions may be associated with the presence of a very mild phenotype.
Supported by FIS 98-556 and Telemarato TV3.

Program Nr: 1632 from the 1999 ASHG Annual Meeting

Mapping of the Fried syndrome gene through a maternally inherited Xp deletion to Xpter-DXS987. *M.J. Dasouki[1], C. Cohick[1], C. Vnencak-Jones[2], V.G. Dev[3].* 1) Sect Medical Gen & Molec Med, Children's Mercy Hosp, Kansas City, MO; 2) Department of Pathology, Vanderbilt University, Nashville, TN; 3) Genetics Associates, Nashville, TN.

Fried syndrome is an X-linked mental retardation (XLMR) syndrome with hydrocephalus, and mild facial dysmorphism. It has considerable similarity to the MASA syndrome which results from mutations in the L1CAM gene in Xq28. Previous molecular studies indicated that this gene lies within the interval KAL-DXS989 in Xp22. Here, we report a maternally inherited Xpter-22.3 deletion found in an 18-year old G1P1 woman and her female infant. Prenatal ultrasonographic examination of the daughter showed massive hydrocephalus due to aqueductal stenosis while her mother had short stature only. FISH analysis using X-WCP (Whole Chromosome Paint) failed to reveal any evidence of a cryptic translocation in either mother or child. In both individuals, the steroid sulfatase and Kallman syndrome gene specific probes were found to be deleted by FISH analysis.X-chromosome inactivation studies performed on peripheral blood lymphocytes using cytogenetic techniques demonstrated that in both mother and daughter, 20/20 cells showed the deleted X-chromosome to be inactive. However, peripheral blood DNA analysis at the HUMARA locus showed exclusive inactivation of the deleted X-chromosome in the mother while the daughter had exclusive inactivation of her normal X-chromosome. Genotyping using fluorescently labeled microsatellite markers that map to the interval DXS6807-DXS6789 (distal 100 cM on Xp) showed biallelic inheritance except for the most distal marker DXS6807. These results indicate that the entire X-chromosome pseudo-autosomal region including the SHOX gene was deleted and that the Fried syndrome gene maps to the distal 13.8 cM of Xp. Additional studies using FISH and microsatellite gentoyping are underway to narrow the deleted region. Further X-chromosome inactivation studies in other tissues may provide a better explanation for the difference in phenotypes and X-inactivation patterns between mother and child.

Program Nr: 1633 from the 1999 ASHG Annual Meeting

Cardioferlin, a novel dysferlin family member and potential modifier of muscular dystrophy. *D.B. Davis[1], E.M. McNally[2]*. 1) Department of Pathology, University of Chicago, Chicago, IL; 2) Department of Medicine, Section of Cardiology, University of Chicago, IL.

Dysferlin was recently identified as the gene mutated in Limb Girdle Muscular Dystrophy type 2B and Miyoshi myopathy (Bashir, et al., Nat Genet 1998 Sep;20(1):37-42 and Liu, et al., Nat Genet 1998 Sep;20(1):31-6). These two muscle disorders vary considerably in phenotypic severity, and the type of mutation found in dysferlin cannot account for these differences. This suggests that additional genetic loci may modify phenotypic severity. Through homology searches, we have identified and cloned a novel cDNA that codes for a protein we have termed cardioferlin. Cardioferlin has been localized to human chromosome 10q24. Cardioferlin, like dysferlin, encodes a 7.5 kb mRNA with an open reading frame encoding a protein of at least 2087 amino acids. The two proteins are 58% identical and 75% similar at the amino acid level. Both proteins contain four C2 domains, which are involved in membrane fusion events. Cardioferlin also contains a unique SH3 domain, implying other protein-protein interactions. Northern blot analysis shows that cardioferlin mRNA is expressed in all tissues, with an eight-fold higher expression level in heart than in skeletal muscle. We have also developed a polyclonal antibody specific for cardioferlin. Cardioferlin is a 230 kD protein found in membrane fractions of heart and skeletal muscle. Due to the high level of similarity between dysferlin and cardioferlin, it is possible that cardioferlin may be able to perform a partial rescue of the loss of dysferlin in LGMD 2B and Miyoshi myopathy explaining the milder spectrum of these muscular dystrophies. Due to the presence of C2 domains in this family of proteins, it is likely that they play a role in membrane repair. We have also demonstrated upregulation of cardioferlin in skeletal muscle membranes from mdx mice lacking dystrophin. Upregulation of cardioferlin may play an important role in the repair of membrane damage seen in some forms of muscular dystrophy. Due to its expression pattern, cardioferlin is a promising candidate as a primary or secondary effector in muscular dystrophy or cardiomyopathy.

Program Nr: 1634 from the 1999 ASHG Annual Meeting

Towards identification of the gene responsible for Acadian Usher Syndrome on 11p. *M. DeAngelis[1,2], S. Savas[2], C. Donaldson[2], L. Buckley[2], J. Doucet[3], C. Visser[2], Z. Den[2], M. Pelias[2], P. Deininger[4], B. Keats[1,2], M. Batzer[1,2].* 1) Neuroscience Ctr., LSU Med Ctr., New Orleans, LA; 2) Dept. of Biometry and Genetics, LSU Med Ctr., New Orleans, LA; 3) Dept. of Biological Sciences, Nicholls State Univ., Thibodaux, LA; 4) Tulane Cancer Ctr., New Orleans, LA.

The Usher syndromes are a group of autosomal recessive disorders characterized by congenital sensorineural hearing impairment and progressive retinitis pigmentosa usually resulting in blindness. Three types of Usher Syndrome have been described and chromosomal locations of nine genetic loci reported, with only two of the genes identified to date. Usher syndrome Type 1, the most severe form, is distinguishable from Usher syndromes types II and III by a severe to profound deafness, absence of vestibular function and an earlier onset of retinal degeneration. Type I Usher syndrome occurs in populations throughout the world, including a small Southwestern Louisiana population descended from French speaking emigrants exiled from Acadia. Our studies focus on the 11p locus for type 1c Usher syndrome (USH1C) in families of Acadian ancestry. Previously, we constructed a contiguous sequnce-ready physical map of bacterial artificial chromosomes (BACs) containing the USH1C critical region using 17 genetic markers. These genetic markers allowed us to refine the critical region by haplotype analysis to a physical distance of less than 400 kb between genetic markers D11S1397 and D11S1890. Sequencing to a 5X coverage of one 150 kb BAC from this region followed by computer-aided analysis yielded 3 genes, that are excellent USH1C candidates. At present we are using RT-PCR and northern blot analysis in order to study the expression pattern of these genes. These genes will then be used for mutational analysis in affected patients in order to identify the gene responsible for USH1C.

Program Nr: 1635 from the 1999 ASHG Annual Meeting

Familial case of Frasier Syndrome indicates that the WT1 KTS donor splice site mutation does not affect ovarian development and function. *L.A. Demmer[1], W.A. Primack[1,2], V. Loik[3], R.S. Brown[1], N. Therville[4], K. McElreavey[4].* 1) Dept Pediatrics, Univ Massachusetts Memorial Health Care, Worcester, MA; 2) Fallon Clinic, Worcester, MA; 3) Department of Obstetrics, Baystate Medical Center, Springfield, MA; 4) Department of Human Immunogenetics, Institut Pasteur, Paris, France.

The description of Frasier syndrome until now has been restricted to XY females with gonadal dysgenesis, progressive glomerulopathy and significant risk of gonadoblastoma. Mutations in the donor splice site in intron 9 of the Wilms' tumor (WT1) gene have been shown to cause Frasier syndrome and are distinct from WT1 exon mutations associated with Denys Drash syndrome. The WT1 gene, which is essential for normal kidney and gonadal development, encodes a zinc finger transcription factor. The intron 9 alternatve splice donor site mutation seen in Frasier syndrome leads to loss of three amino acids (+KTS isoform), thus disrupting the ratio of the +KTS/-KTS isoforms critical for proper gonadal and renal development.

Here we report two sisters with identical intron 9 mutations (cytosine-thymidine transition at position +4 of the donor splice site). The proband carries a classic diagnosis of Frasier syndrome with focal segmental glomerulosclerosis (FSGS) and 46,XY gonadal dysgenesis while her sister has the progressive glomerulopathy but a 46,XX karyotype and normal female development. This demonstrates that the proper WT1 isoform ratio is critical for renal and testicular development, but does not affect either ovarian development or function.

We propose that the clinical definition of Frasier syndrome should be broadened to include 46,XX females with normal genital development and FSGS associated with a WT1 intron 9 donor splice site mutation. The risk for gonadal malignancy in these patients as well as the risk for kidney disease and gonadal dysgenesis in their offspring needs to be considered.

Program Nr: 1636 from the 1999 ASHG Annual Meeting

A truncation mutation (L126Z) of SOD1 gene leads to ALS-like phenotype in transgenic mice. *H-X. Deng, R. Fu, H. Zhai, T. Siddique.* Neurology Dept, Northwestern Univ, Chicago, IL.

Amyotrophic lateral sclerosis (ALS) is a paralytic disorder caused by degeneration of motor neurons in the brain and spinal cord. Mutations in SOD1 gene account for approximately 20% of familial ALS cases. The SOD1-knockout mice develop normally and the transgenic mice expressing several missense mutations of SOD1 gene develop ALS-like phenotype, suggesting a gain of toxic function mechanism underlines the disease. How mutations in SOD1 gene cause Familial ALS and which portion of SOD1 polypeptide is responsible for its toxicity are unknown. We previously described a familial ALS patient with a truncation mutation in SOD1 (L126Z), i.e. a stop codon replaced codon for Leu at 126, truncating the other 28 amino acids at C-terminus. The truncated SOD1 is not detectable in the erythrocytes and lymphoblastoid cells of the patient. To identify the selective expression of truncated SOD1 and further investigate the pathogenesis using this unique mutation, we have developed a transgenic mouse model that expresses this truncated SOD1. These transgenic mice develop an ALS-like phenotype. The symptoms are detectable around 280~320 days and the aniamls die 2~3 weeks later. These results provide proof of principle that a merely shortened version of the SOD1 protein is sufficient to cause disease and indicate that 1) the full length SOD1 is not a requirement for the development of ALS; 2) the C-terminal portion (28 amino acids) is not involved in the toxicity; 3) the N-terminal portion (125 amino acids) is responsible for the toxicity that causes motor neuron death in ALS. Further characterization of this mouse model may provide clues to the pathogenesis of this fatal disease.

Program Nr: 1637 from the 1999 ASHG Annual Meeting

Phenotypic Variability in a Large Aniridia Pedigree with a Novel *PAX6* 1410delC Mutation. *J.L. Dickinson[1], J.E. Craig[2], M. Sale[1], S. Nicholls[1], J. Love[3], I. Hanson[3], S.J. Matthews[2], D.A. Mackey[1,2].* 1) Menzies Centre, University of Tasmania, Australia; 2) Centre for Eye Research Australia, University of Melbourne, Australia; 3) MRC Human Genetics Unit, Edinburgh, Scotland.

Purpose: To investigate phenotypic variability with a mutation in the *PAX6* gene. **Methods:** 11 affected individuals in a 3 generation pedigree were examined. Anterior segment and fundus photographs, automated perimetry and IOP measurement were performed. **Results:** Mutation analysis revealed a single base deletion 1410delC in the *PAX6* gene. Affected individuals ranged from total aniridia to minimal anterior segment findings. Other findings included keratitis, cataract, glaucoma, disc anomalies and foveal hypopolasia. **Conclusions:** A novel mutation in the *PAX6* gene is associated with considerable phenotypic variation. Pedigrees such as this may be useful to identify other genes responsible for anterior segment development.

Program Nr: 1638 from the 1999 ASHG Annual Meeting

Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) : familial report with thymidine phosphorylase mutation. *V. Dideberg[1], A. Verloes[1], B. Sadzot[2], M. Reznik[3], A. Rotig[4], I. Nishino[5], M. Hirano[5]*. 1) Human Genetics, Liege University Hospital, Liege, Belgium; 2) Department of Neurology, Liege University Hospital, Liege, Belgium; 3) Department of Neuropathology, Liege University Hospital, Liege, Belgium; 4) Research Unit on Children's Genetic Handicaps, Hopital Necker-Enfants-Malades, Paris, France; 5) Dept of Neurology, Columbia-Presbyterian Medical Center, New York, USA.

Our proband, born to remotely related parents, was a 30 year-old woman who was initially diagnosed as HSMN type I. Because of probable Guillain-Barr syndrome, she was referred to the University Hospital. She was 172 tall for 42 kg. She presented with severe amyotrophy, abolished reflexes, external ophthalmoplegia, pes cavus, and chronic diarrhea. Investigations showed slow conduction velocities, high frequency deafness, delayed somesthetic EP, and generalized leucoencephalopathy without intellectual impairment. Nerve biopsy confirmed demyelinization. Muscular mtDNA revealed heteroplasmic deletion of about 9 kB, and normal respiratory chain function, allowing a firm diagnosis of mitochondrial neurogastrointestinal encephalomyopathy (MNGIE). A brother of our proband was similarly affected. MNGIE (or POLIP syndrome) is a disorder characterized by chronic intestinal pseudo-obstruction, ophthalmoplegia, demyelinating sensori-motor neuropathy, leucodystrophy, and abnormal muscle (abnormal mitochondria and/or ragged red fibers) associated with mitochondrial dysfunction (complex 4 and/or complex 1 defect) and usually multiple mtDNA deletions (Hirano, Neurology 1994,44,721-727). Surprisingly, MNGIE was shown to be a genetically homogeneous, recessively inherited disorder mapping to 22q13.32-qter (Hirano, AJHG 1998,63,526-533), due to mutations in the thymidine phosphorylase gene (Nishino, Science 1999,283,689-692). In our proband, a homozygous A3371C (E289A) missense mutation in a highly conserved glutamic acid was found in the 7th exon. This appears to be a recurrent mutation, already observed in patients of British, European American and Ashkenazi origin.

Program Nr: 1639 from the 1999 ASHG Annual Meeting

Genetic analysis of tauopathies: corticobasal degeneration shares the same genetic background with progressive sopranuclear palsy. *E. Di Maria[1], T. Vigo[1], M. Tabaton[2], E. Bellone[1], R. Marchese[2], P. Montagna[3], D. Munoz[4], P. Pramstaller[5], G.L. Zanusso[5], N. Rizzuto[5], F. Ajmar[1], P. Mandich[1]*. 1) Dept. of Oncology, Biology and Genetics, Univ. of Genova, Italy; 2) Dept. of Neurological Sciences, Univ. of Genova; 3) Univ. of Bologna, Italy; 4) Univ. of Ontario, Canada; 5) Univ. of Verona, Italy.

Corticobasal degeneration is a rare disease involving cerebral cortex as well as extrapyramidal structures, with clinical signs as apraxia, tremor and rigidity. CBD is included in a group of neurodegenerative diseases characterised by abnormal deposits of protein tau (microtubule associated protein tau, MAPT). This group, named "tauopathies", comprises CBD, progressive supranuclear palsy (PSP), and some forms of frontotemporal dementia (FTD). Missense mutations of MAPT gene were reported in familial cases of FTD; conversely, an association between PSP and the A0 allele of a dinucleotide polymorphism in the intron 9 of the gene was found in several series of patients. When seen at the ultrastructural level, CBD and PSP share the same features of the tau deposits, thus differing from FTD. We recruited to date twenty-two patients affected with CBD and fourteen with FTD. Mutation analysis of MAPT gene coding region did not reveal any mutation; five single nucleotide polymorphisms (SNP) of exon 9 were detected both in patients and controls. In order to characterise the genetic background underlying CBD, fourteen cases were genotyped for the dinucleotide polymorphism in the intron 9: 82% of CBD cases carry the A0 allele, compared to 67% in controls. The association study was therefore extended to a panel of polymorphisms lying in the critical region of tau. A haplotype, resembling the one reported for PSP, is over-represented in CBD, compared with controls as well as with FTD ($p<0.05$). Based on this result, we could conclude that, besides similar clinical and neuropathological features, CBD and PSP share the same genetic background and therefore appear to be the same entity, quite distinct from FTD. Further studies are in progress to investigate the specific genetic defect underlying corticobasal degeneration.

Program Nr: 1640 from the 1999 ASHG Annual Meeting

Heterogeneity within subgroups of the autosomal recessive limb girdle muscular dystrophy in Turkey. *P. Dincer[1], Z. Akcoren[2], E. Demir[2], I. Richard[4], O. Sancak[1], G. Kale[2], S. Ozme[2], E. Tan[3], J.A. Urtizberea[5], J.S. Beckmann[4], H. Topaloglu[2].* 1) Medical Biology, Hacettepe University,, Ankara, TURKEY; 2) Children's Hospital; 3) Neurology; 4) Genethon, Evry, France; 5) Institute de Myology-AFM,Groupe Hospitalier Pitie-Salpetriere, Paris,France.

Autosomal recessive limb girdle muscular dystrophies (LGMD2) represent a heterogeneous group of diseases. A broad range of clinical and genetic entities is observed, ranging from very mild to severe forms and from LGMD2A to LGMD2H subgroups. Eight genes were mapped for AR-LGMD2s; they are LGMD2A (CAPN3) at 15q, LGMD2B (dysferlin) at 2p, LGMD2C (g-SG) at 13q, LGMD2D (a-SG) at 17q, LGMD2E (b-SG) at 4q, LGMD2F (d-SG) at 5q, LGMD2G at 17q and LGMD2H at 9q. We have evaluated 38 LGMD2 families with 65 cases by linkage analysis for known loci of LGMD2A to LGMD2F, and protein studies. All families are Turkish. Consanguinity was present in 37 of them. The classification of the families according to the combined data of linkage and protein analysis was as follows; calpainopathy 7, dysferlinopathy 3, g-sarcoglycan deficiency 5, a-sarcoglycan deficiency 2, b-sarcoglycan deficiency 7, d-sarcoglycan deficiency 1 and merosinopathy 2. There were two families showing an Emery-Dreifuss phenotype with normal staining of Emerin and 9 showing no linkage to the LGMD2A to F loci, and they had normal sarcoglycans. There is extreme heterogeneity in the clinical presentation. g-sarcoglycan deficiency seems to be the most severe group as a whole, whereas dysferlinopathy is the mildest. In sarcoglycan deficiencies, sarcoglycans other than the primary ones may also be reduced severely, however this may not reflect to the phenotype. Many cases of primary g-sarcoglycan deficiency stained normal or only mildly abnormal d-sarcoglycan staining. Calf hypertrophy is a cardinal feature of sarcoglycan deficiencies. Interfamilial variability of symptoms was a common finding in most groups. Cardiomyopathy was not present in any of the families during evolution. Scapular winging and selective wasting of hamstrings is peculiar for calpainopathy. Support: TUBITAK (SBAG-1774), Turkey and AFM, France.

Program Nr: 1641 from the 1999 ASHG Annual Meeting

Gene discovery in Acadian Usher Syndrome. *J.P. Doucet[1], A.R. Clement[1], L.C. Ledet[1], S.S. Robichaux[1], J.R. Landry[1], S.M. Robichaux[1], M.M. DeAngelis[2], B.J.B. Keats[3], M.A. Batzer[4], P.L. Deininger[5].* 1) Molecular Genetics Section, Biological Sciences, Nicholls State University, Thibodaux, LA; 2) LSU Neuroscience Center, New Orleans, LA; 3) Department of Biometry and Genetics, LSU Medical Center, New Orleans, LA; 4) Department of Pathology, LSU Medical Center, New Orleans, LA; 5) Tulane University Cancer Center, New Orleans, LA.

The Usher Syndromes are the most common multisensory deficits known. Type I Usher Syndrome, characterized by profound deafness, vestibular dysfunction, and retinal degeneration, segregates in a small population of Acadian descendants deriving from southwestern Louisiana. Of the nine loci linked to the Usher Syndromes, only 11p14.2-15.1 is linked to the Acadian population. In an effort to discover the causative gene in Acadian Usher Syndrome (USH1C), we have obtained partial nucleotide sequence of our previously reported bacterial artificial chromosome contig (DeAngelis, et al., Biochim Biophys Acta 1407 [1998]). Through nucleotide similarity analyses, we have identified several putative exons that map to the USH1C critical region. Using primers designed to the internal aspects of these putative exons, we have determined by amplification the existence of these exons in various cDNA sources and have identified one exon that appears in retinal cDNA. Using the RACE (Rapid Amplification of cDNA Ends) technique, we have derived a 3-kb gene fragment from this retinal exon that presumable contains the full 5 end of its coding sequence. This cDNA is unique from others our group has discovered. The discovery of a retinally-expressed sequence in the USH1C critical region makes its gene a prime candidate for the USH1C gene.

Program Nr: 1642 from the 1999 ASHG Annual Meeting

Real-time quantitation of the relative levels of Dp260 mRNA expression in mouse tissues reveals an abundance in eye as compared to brain, heart and skeletal muscle. *S.J. Dwinnell[1], N.M. Duncan[2], S.A. Tokarz[1], P. Pattee[2], D.M. Pillers[1,2]*. 1) Dept Molecular & Medical Gen, Oregon Health Sci Univ, Portland, OR; 2) Dept Pediatrics, Oregon Health Sci Univ, Portland, OR.

Purpose: Duchenne muscular dystrophy (DMD) results from mutations within the X-linked *DMD* gene. In addition to skeletal myopathy, patients may exhibit non-muscle manifestations such as cognitive dysfunction, cardiomyopathy, and abnormalities of retinal electrophysiology. The *DMD* gene encodes full-length dystrophin (Dp427) and four smaller isoforms that all conserve the carboxyl terminus of Dp427: Dp260, Dp140, Dp116, and Dp71. Dp260 was first identified in retina and genotype/phenotype correlation studies in DMD/BMD patients and *dmd* mouse models show that mutations disrupting Dp260 result in abnormal retinal electrophysiology. We hypothesized that *DMD* gene defects which alter Dp260 expression in retina, heart and brain may cause non-muscle manifestations of DMD/BMD, and sought to determine the relative extent of Dp260 mRNA expression in mouse. **Methods:** The TaqMan and ABI 7700 system (Perkin Elmer) was used to measure Dp260 transcript levels relative to that of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) in each tissue by real-time quantitative RT-PCR. PCR primers and fluorogenically labeled probes for mouse Dp260 and GAPDH cDNA sequences were designed using Primer Express software (Perkin Elmer). mRNA was isolated from brain, eye, heart and skeletal muscle of C57BL/6J mice and cDNA was synthesized using sequence-specific reverse PCR primers. PCR reactions were performed on the ABI 7700 sequence detection/thermocycler. Relative levels of Dp260 fluorescence emission were determined in real-time and compared to GAPDH for each tissue. **Results:** The ratio of Dp260/GAPDH was approximately equal in brain, heart and skeletal muscle, but was significantly increased in eye as compared to the other tissues. **Conclusion:** The finding that Dp260 is more highly expressed in eye than in skeletal muscle and other non-muscle tissues is consistent with the genotype/phenotype correlation studies that implicate Dp260 as playing a crucial role in normal retinal electrophysiology in the mouse.

Program Nr: 1643 from the 1999 ASHG Annual Meeting

Isolation and characterization of a novel candidate gene, SMT7, in the facioscapulohumeral muscular dystrophy (FSHD) gene region. *J.T. Ehmsen[1], J.D. Forrester[2], J.E. Hewitt[3], D.A. Figlewicz[2], S.T. Winokur[1]*. 1) University of California , Irvine, CA; 2) University of Rochester, Rochester, NY; 3) Nottingham University, Nottingham, UK.

Facioscapulohumeral muscular dystrophy (FSHD) is the third most common form of inherited muscle disease following Duchenne and myotonic dystrophy. FSHD is initially characterized by an asymmetric, progressive weakness of the facial and pectoral girdle muscles. The disorder is inherited in an autosomal dominant fashion with nearly complete penetrance and results from a deletion of integral copies of a heterochromatic 3.3 kb tandem repeat unit on the long arm of chromosome 4 (D4Z4). A "short" EcoR1 fragment (< 35 kb) containing D4Z4 segregates with the disease, while the size of this polymorphic locus in the normal population ranges between 35 and 300 kb. The specific gene(s) responsible for FSHD have not been identified. However, integral deletions of the heterochromatic D4Z4 repeat appear to disrupt the normal expression of genes proximal to D4Z4, a phenomenon akin to position effect variegation in Drosophila and telomere silencing in yeast. We have cloned and characterized a novel gene which maps to the FSHD gene region. This gene, SMT7, was initially identified in the Unigene '97 EST database as mapping to distal 4q. We have refined its map position within 4q35 through a radiation hybrid minipanel and determined that it maps proximal to the D4Z4 repeat array adjacent to the skeletal muscle specific gene ALP. SMT7 is expressed in adult skeletal muscle, as determined by RT-PCR. Interestingly, expression of SMT7 gene is increased in FSHD muscle tissue compared to control muscle, suggesting a position effect involving this gene in FSHD. Using 20 ng of muscle cDNA per sample, we carried out RT-PCR on 8 control samples and 10 FSHD samples. The gene was expressed, on average, 3X higher in FSHD muscle samples than controls. Sequence analysis on 950 bp of this transcript reveals no significant homologies to known genes. We are currently sequencing two skeletal muscle cDNA clones which span an additional 2.5 kb of the SMT7 transcript. Studies are also underway to map and isolate the mouse homolog.

Program Nr: 1644 from the 1999 ASHG Annual Meeting

Mutation screening for the calcium channel beta subunit gene *CACNB4* in familial ataxia and epilepsy. *A. Escayg[1], D.D. Lee[1], R. Baloh[2], T. Sander[3], M.H. Meisler[1]*. 1) Dept Human Genetics, Univ Michigan, Ann Arbor 48109-0618, MI; 2) Dept Neurology, UCLA, Los Angeles; 3) Dept Neurology, U. Hospital Charite, Humboldt U., Berlin.

Four paralogous mammalian genes encode the cytoplasmic beta subunits of the voltage gated calcium channel. A loss-of-function mutation in the beta 4 subunit, *CACNB4*, was identified in 1997 in the mouse neurological mutant *lethargic*. Homozygous *lethargic* mice exhibit ataxic gait and seizures, suggesting that *CACNB4* may be considered a candidate gene for the corresponding human disorders. To test this possibility, we determined the exon/intron structure of human *CACNB4* (Genomics 50:14) and designed PCR primers to amplify the exons from genomic DNA. The genomic PCR products are screened for mutations by SSCP and conformation sensitive gel electrophoresis (CSGE). Mobility variants are gel purified and manually sequenced. Two patient populations are being analyzed. Families with seizures were ascertained through members with childhood absence epilepsy (CAE), juvenile absence epilepsy (JAE), or juvenile myoclonic epilepsy (JME) (Epilepsy Res. 29:115). The second group of patients exhibit hereditary episodic ataxia. DNA samples from 255 controls are also being tested. Two potential disease mutations are under investigation. Eight silent SNPs with frequencies between 0.001 and 0.026 have also been identified. Allele specific oligonucleotides (ASOs) and diagnostic restriction digests have been developed for analysis of these SNPs in association and linkage studies.

Program Nr: 1645 from the 1999 ASHG Annual Meeting

Variability in the PS deficient phenotype associated with *PROS1* allelic heterogeneity. *Y. Espinosa-Parrilla[1], M. Morell[1], M. Borrell[2], J.C. Souto[2], J. Fontcuberta[2], X. Estivill[1], N. Sala[1].* 1) Departament de Genètica Molecular, I.R.O, Barcelona, Spain; 2) Unitat d'Hemostasia i Trombosis, Hospital de Sant Pau, Barcelona, Spain.

Anticoagulant protein S (PS) deficiency is a cause of thrombophilia. To study the molecular basis of its phenotypic heterogeneity, the results of *PROS1* gene and mutation analysis were compared to the PS values in 54 families with different types of PS deficiency and thrombosis. Type I PS deficiency (reduction of total and free PS antigen) was found in 18 families and all but one family that segregated the rare P460 allele of the S/P460 variant, had a cosegregating mutation. Type III deficiency (only free PS antigen is reduced) was found in 23 families in which the unique *PROS1* alteration was the presence of 3 rare allele variants in 3 families and the P460 allele in 11 families (47.8%), though it did not always segregate with the deficiency. Finally, 13 families were classified as type I/III since both phenotypes coexisted in these families. Six of them carried a cosegregating mutation, 2 had a non cosegregating variant and 3 segregated the P460 allele. Mean free PS levels in type III individuals were significantly higher ($p<0.0001$, unpaired t-test) than those in type I, and mean total PS levels in type III individuals were significantly lower ($p<0.0001$) than those in normal relatives. In the pedigrees segregating the S/P460 variant, not only free but also mean total PS levels were found significantly lower in the SP460 heterozygotes than in the SS460 homozygotes. This, together with the finding of a P460 homozygote with type I deficiency, suggests a role for this PS variant in the deficient phenotype. Besides, in vitro expression analysis, performed with 6 *PROS1* missense mutants, showed differential secretion of these mutants causing phenotypic heterogeneity in PS deficiency. All together indicating that while type I is a quantitative PS deficiency, essentially due to different detrimental *PROS1* mutations, type III deficiency is a milder quantitative deficiet phenotype caused by some functional *PROS1* variants and other genetic and/or environmental factors that determine variability in PS levels.

Program Nr: 1646 from the 1999 ASHG Annual Meeting

New mutations in the voltage-gated calcium channel gene CACNA1A in british families with episodic ataxia type 2. *L.H. Eunson[1], A. Jouvenceau[1], V. Ramesh[2], S.M. Zuberi[3], A. Nairne[4], N. Hyman[4], N.W. Wood[1], A. Spauschus[1], D.M. Kullmann[1], M.G. Hanna[1]*. 1) Clinical Neurology, Institute of Neurology, Queen Square London, England; 2) Newcastle General Hospital; 3) Royal Hospital for Sick Children, Glasgow; 4) Battle Hospital, Reading.

Episodic ataxia type 2 (EA2) is an autosomal dominant disorder caused by mutations in the voltage-gated calcium channel gene, CACNA1A, on chromosome 19q. We have identified fifteen new families with the EA2 phenotype. We have analysed exons 4,6,16,17,22,24 and 36 that have previously been reported to harbour pathogenic mutations, and have also analysed exons 2,3,7,11,12,25,26,28,30,31,38,41,42 and 43 by SSCP and DNA sequencing. We have determined the length of the CAG repeat using fluorescent PCR techniques. They were normal in all cases. None of the EA2 probands harboured any of the previously reported mutations in these exons. In two families we identified novel pathogenic mutations. The proband in family 1 exhibited typical attacks of EA2, but in addition had absence epilepsy and learning difficulties. He was unresponsive to acetazolamide. We identified a heterozygous C to T transition at nucleotide 5733 in exon 36 (CGA to TGA resulting in R1820stop). This predicts the introduction of a premature stop codon at the beginning of the C terminus of the alpha 1A subunit. Affected members of family 2 exhibited typical acetazolamide-responsive EA2. We identified a heterozygous intronic 4 base pair deletion at +3 of intron 41. This mutation is predicted to disrupt channel function either by causing exon skipping or alternative splicing. Both mutations were not detected in over 100 control chromosomes, have not been reported previously, and are likely to be pathogenic. The R1820stop mutation is the most distal truncation mutation identified in CACNA1A and suggests that loss of the C terminal region may be the important functional consequence of other truncation mutations described. An intronic deletion mutation has not been reported previously in CACNA1A and suggests a new mutational mechanism. The absence of known mutations in the other families suggests further genetic heterogeneity associated with the EA2 phenotype.

Program Nr: 1647 from the 1999 ASHG Annual Meeting

Additional *CDMP1* mutations suggest that brachydactyly type C is locus homogeneous and incompletely penetrant. *D.B. Everman[1], A. Polinkovsky[1], C.A. Francomano[2], J.M. Graham, Jr.[3], F.R. Goodman[4], G. Neri[5], S. Morrison[1], N.H. Robin[1], M.L. Warman[1]*. 1) Case Western Reserve Univ and Univ Hospitals, Cleveland, OH; 2) NHGRI, NIH, Bethesda, MD; 3) Cedars Sinai Medical Center, Los Angeles, CA; 4) Institute of Child Health, London, UK; 5) Univ Cattolica, Rome, Italy.

We have previously reported that mutations in the TGF-b superfamily member *CDMP1* can cause autosomal dominant brachydactyly type C (BDC). Herein we describe additional *CDMP1* mutations confirming that *CDMP1*, on human chromosome 20, is the major BDC locus and that the most common mechanism of mutational effect is functional haploinsufficiency. Also, in two separate kindreds, we detected mutation carriers who lacked findings of BDC, indicating that functional haploinsufficiency mutations may be non-penetrant. In an English kindred, an affected mother transmitted a mutant *CDMP1* allele to two of her children; her daughter has typical BDC while her son appears clinically unaffected and radiologically has a normal metacarpal-phalangeal profile. This mutation co-segregates with the disease phenotype in the mother's extended family. In an American kindred, an unaffected (clinically and radiographically) mother, whose son has typical features of BDC, was also found to have a *CDMP1* mutation; we have not yet determined whether the mother's mutation was inherited, or arose *de novo*, in which case somatic mosaicism could explain her non-penetrance. Interestingly, both families have the same mutation, an insertion G at nucleotide residue 206 of the coding sequence which creates a reading frame shift beginning at amino acid residue 69 and causes premature termination 25 amino acid residues downstream. This mutation has not been detected in over 100 control alleles. We have also identified a *CDMP1* mutation in the kindred with BDC that was originally described by Haws; linkage analysis in this kindred had suggested the existence of a second BDC locus on human chromosome 12q. However, we found a 23 bp insertion within the *CDMP1* coding sequence that co-segregates with the family's phenotype. This indicates that BDC is most likely locus homogeneous and that prior linkage to chromosome 12q can be attributed to type I error.

Program Nr: 1648 from the 1999 ASHG Annual Meeting

Search for X-Chromosomal Microdeletions in Rett Syndrome. *F. Fan[1], R.E. Amir[3], X. Zhang[2], E.J. Dahle[3,4], H. Zoghbi[3,4], U. Francke[1,2]*. 1) Dept. of Genetics; 2) Howard Hughes Medical Institute, Stanford Univ Sch of Med, Stanford, CA; 3) Dept. of Pediatrics and Molecular and Human Genetics; 4) Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX.

Rett Syndrome (RTT) is a neurodevelopmental disorder that affects mostly females. Symptoms first appear at 6-18 months of age with progressive loss of acquired language and fine motor skills and development of stereotypical hand movements. Severe mental impairment is usually present. The estimated prevalence of 1 in 15,000 females makes it one of the more common causes of mental disability. The majority of the cases are sporadic, presumably due to independent new mutations. In an effort to delineate the genetic basis of RTT, we tested the hypothesis that RTT may be caused by recurrent microdeletions of the X chromosome which are not readily detected by cytogenetic studies. Such microdeletions could be mediated by region-specific duplications leading to non-homologous recombination events, analogous to the autosomal Williams and Prader-Willi/Angelman syndromes. We studied a panel of somatic cell hybrids from 7 affected RTT females representing 5 independent mutational events. Either the maternal or the paternal X chromosome, but not both, was retained in these hybrid cell lines. Therefore, we were able to assay STS markers by PCR. We also included cells from a severely affected male carrying a familial Rett mutation (Schanen and Francke, AJHG 63:267,1998). Our deletion survey covered about 35Mb of the X chromosome that were not excluded from carrying the RTT gene at the time we initiated our study. PCR assays were established for a total of 425 mapped STS/EST markers. For the Xp22 region DXS85-DXS1053, 50 markers were tested for an average inter-marker distance of 142kb. This region was subsequently excluded by family studies. On the long arm, for region DXS1206-DXS1227 (Xq25-q27) 238 markers were tested and for region DXS998-DXS7062 (Xq27-q28) there were 137 markers, with the average distance between the long arm markers being 75 kb. No microdeletions were found in our test panel. We conclude that a microdeletion of 100kb or larger is not likely to be a common mutational mechanism for RTT.

Program Nr: 1649 from the 1999 ASHG Annual Meeting

Linkage exclusion in nine families with probable Parkinson's disease. *M.J. Farrer[1], A. Destée[2], E. Becquet[2], F. Wavrant-De Vriéze[1, 3], V. Mouroux[3], F. Richard[3], L. Defebvre[3], S. Lincoln[1], J. Hardy[1], P. Amouyel[3], M-C. Chartier-Harlin[3].* 1) Neurogenetics, Mayo Clinic Jacksonville, Jacksonville, FL; 2) Clinique Neurologique, CHRU, 59037 Lille Cedex France; 3) INSERM 508, Institut Pasteur de Lille, 59019 Lille Cedex, France.

We have analyzed the segregation of genetic markers spanning chromosomal regions 2p13, 4p14-15, 4q21-23, 6q25-27 and 17q21 in nine French families affected by autosomal dominant probable Parkinsons disease. These regions have been linked/associated with familial Parkinsons disease or parkinsonism. Multipoint linkage and haplotype analysis excluded 2p13 and 4p14-15 loci in 4/9 and 5/9 kindreds respectively. For four families, which were equivocal for linkage to 4p14-15 (D4S405), the ubiquitin carboxy-terminal hydrolase gene (UCH-L1) was sequenced. In one family, a novel UCH-L1 M124L mutation that did not segregate with early-onset disease was identified. This suggests that rare variants in this gene may not be pathogenic. In 7/9 families, it could be inferred that affected individuals did not share 4q21-23 (alpha-synuclein) haplotypes. All families were unequivocally excluded, by haplotype analysis, from the parkin locus on 6q25-27. Finally, the 17q21 region was excluded in 5/9 kindreds and no mutation in the tau gene was identified in the four remaining families. Findings from this study confirm and extend the observation that familial parkinsonism is genetically heterogeneous.

Program Nr: 1650 from the 1999 ASHG Annual Meeting

Neonatal diagnosis of Cystic Fibrosis: Application of the Elucigene™CF20 test using blood spots samples. *D. Feldmann, C. Magnier, L. Vallat, C. Houdayer, M. Tredano, M. Pressac, P. Aymard.* Service de Biochimie, Hopital Trousseau, Paris, France.

DNA analysis of neonatal samples for CF is becomming more widespread and there is a need for a cost-effective method wich can detect many mutations and is suitable for blood spots. ARMS (Amplification Refractory Mutation System) technology has been applied for mutation detection. We evaluated a newly developped multiplex ARMS test wich detects 20 mutations in the CFTR gene to assess its suitability for routine analysis of blood spots samples in a clinical laboratory. 26 samples with elevated immunoreactive trypsinogen levels were selected for DNA analysis. DNA was extracted from the blood spots samples by a rapid alkali lysis method and directly added to 3 multiplex ARMS reactions. Following PCR amplification, the products of each reaction were analysed by agarose gel electrophoresis. All DNA were successfully amplified using the test including samples stored fot a period of up to one year or more. We detected mutations in 6 of the 26 samples analysed. The test results were concrdant with data obtained using other mutation detection methods according to the limitation of the test (homozygosity information for DF508 only). Results could be obtained in one day. We conclude that the test is a simple and rapid screening method for molecular analysis of the CFTR gene in neonates.

Program Nr: 1651 from the 1999 ASHG Annual Meeting

Identification of the gene responsible for non-type I cystinuria, *SLC7A9*. *L. Feliubadaló[1,5], The Cystinuria Consortium[1,2,3,4,5]*. 1) Dpt. Genètica Molecular, IRO, L'Hospitalet Ll, Barcelona, Spain; 2) Dpt. Medicine C, Sheba Medical Center, Tel Hashomer, Israel; 3) SGM, Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy; 4) TIGEM, San Raffaele Biomedical Science Park, Italy; 5) Dpt. Bioquímica i Biologia Molecular, Fac. Biologia, Universitat de Barcelona, Spain.

Cystinuria (OMIM 220100) is a common recessive disorder of renal and intestinal re-absorption of cystine and dibasic aminoacids. Mutations in *SLC3A1*, encoding rBAT, produce cystinuria type I. A gene causing non-type I cystinuria has been assigned by linkage analysis to 19q12-13.1. We have identified a kidney cDNA (*SLC7A9*) of the family of the light subunits of amino acid transporters, expressed in kidney, liver, small intestine and placenta, and its gene is localized to the non type I cystinuria 19q locus. Cotransfection of the SLC7A9, encoding $b^{o,+}$AT, and *SLC3A1* brings the latter to the plasma membrane and results in the expression of L-arginine uptake in COS cells. We have found *SLC7A9* mutations in Libyan-Jewish, North American, Italian and Spanish non type I cystinuria patients. The Lybian-Jewish patients are homozygous for a founder mutation which abolishes $b^{o,+}$AT amino acid uptake activity when cotransfected with rBAT in COS cells. Four missense, two in homozygous state, and two frameshift mutations were identified in other patients. Our data establish that mutations in *SLC7A9* cause non-type I cystinuria, and strongly suggest that $b^{o,+}$AT is the light subunit of rBAT.

Program Nr: 1652 from the 1999 ASHG Annual Meeting

An explanation for the origin and pathogenic role of the hereditary pancreatitis-associated N21I mutation in the cationic trypsinogen gene. *C. Ferec, J.M. Chen.* Centre de Biogenetique, Universite, Hopital, ETSBO, Brest, France.

Hereditary pancreatitis (HP) is an autosomal dominant disease. Two missense mutations-an R117H in exon 3 and an N21I in exon 2 of the cationic trypsinogen gene have been associated with HP. While the R117H mutation could be explained by disrupting an important "self-destruct" mechanism, why and how the N21I substitution could cause a "gain of function" of the cationic trypsin remains one of the most important but unanswered questions. We address this issue by pursuing the origin of this missense mutation in the context of the human trypsinogen gene family and then unify the current available knowledge to explain its pathogenic role in HP. There is strong evidence suggesting that the N21I substitution arose as a gene conversion event in which the wild-type functional anionic trypsinogen gene acted as the donor sequence: the only presence of Ile at residue 21 of the anionic trypsinogen gene amongst the several trypsinogen genes; a high overall sequence homology between the cationic and anionic genes; a single unbroken tract of nucleotides of up to 113 bp flanking the I21 residue in the anionic gene; a chi-like sequence present in the 5' proximity and a perfect seven nucleotide inverted repeat split by an asymmetric 21 bp sequence located in the 3' proximity of the mutation. A multiple alignment of the partial sequences around residue 21 of vertebrate trypsins further indicated that N21 and I21 represent rare mutations of the two functional human trypsinogen genes, positively selected by evolution to somehow endow an as yet unknown advantageous effect on their respective protein's structure and function. Thus, a non-reciprocal genetic information exchange between them at this position could be disastrous. In support of this, the physico-chemical properties of Ile are quite different from Asn and a structural difference of this substitution has been suggested by different affinity constants of a monoclonal antibody towards the two trypsinogens and by computer prediction analysis. These observations, taken together, enabled us to complement a working model for the N21I mutation, which highlights an indirect impairment on the "self-destruct" mechanism.

Program Nr: 1653 from the 1999 ASHG Annual Meeting

Russell-Silver syndrome: Identification of a candidate region on the short arm of chromosome 7. *B.A. Fernandez[1], I. Teshima[1], K. Nakabayashi[1], V. Proud[2], R. Weksberg[1], S.W. Scherer[1].* 1) Dept. of Genetics, Hosp Sick Children, Univ Toronto, Toronto, ON, Canada; 2) Valley Children's Hosp, Madera, CA.

Russell-Silver syndrome (RSS) is a form of dwarfism characterized by IUGR, triangular facies and 5th finger clinodactyly. Ten percent of sporadic cases are associated with maternal uniparental disomy for chromosome 7 (matUPD7), suggesting that the etiology in some patients is an alteration in one or more imprinted genes on chromosome 7.

Two RSS patients were identified, each with a cytogenetic rearrangement of 7p. Patient 1 had a partial duplication of 7p [46,XX,dup(7)(p12p14)]. Patient 2 had a paracentric inversion, with the proximal inversion breakpoint at 7p14 [46,XY,inv(7)(p14p21)]. We hypothesized that the RSS phenotype in both patients might be due to alteration in an imprinted gene or region on 7p. Also, given the previous matUPD7 data, we hypothesized that patient 1's duplication was carried on her maternal chromosome 7.

The first hypothesis was tested by fluorescence *in situ* hybridization (FISH) mapping. Metaphase FISH experiments using YACs and PACs for the 7p region were performed. Results indicate that patient 1's duplication extends from 7p12 into 7p14. In patient 2, a YAC that spans the proximal (7p14) inversion breakpoint, is also duplicated in patient 1. Thus, a common region of involvement (1 Mb in size) on 7p14 has been identified, and we propose this as a possible RSS candidate region.

In patient 1, the parent-of-origin of the chromosome carrying the duplication was determined by typing 8 fluorescent short tandem repeats (STRs) in the patient and her parents (5 STRs from within the duplicated region and 3 STRs from the flanking regions). Five quantitative multiplex PCR reactions have confirmed that patient 1's duplication is carried on her maternal chromosome 7. This finding suggests that RSS in patients with matUPD7 is due to excess of a maternally expressed growth-suppressing gene, rather than due to deficiency of a paternally expressed growth-promoting gene.

Program Nr: 1654 from the 1999 ASHG Annual Meeting

Quantitative Analysis of 4q35 Genes. *J. Forrester[1], R. Morsch[1], J.E. Sowden[1], R.C. Griggs[1], R. Tawil[1], D.A. Figlewicz[1,2].* 1) Neurology, University Rochester, Rochester, NY; 2) Neurobiology and Anatomy, University of Rochester, Rochester, NY.

Facioscapulohumeral muscular dystrophy (FSHD) is the third most common form of muscular dystrophy, resulting from deletions of integral copies of a 3.3Kb repeated element, known as D4Z4, on chromosome 4q35. However, no transcripts have been isolated from D4Z4, or from the distal subtelomeric DNA. Current experimental work is centered around a hypothetical mechanism: disruption of gene expression at 4q35 may result from telomeric chromatin reorganization due to these deletions. We are using quantitative competitive RTPCR to determine expression levels of genes which lie centromeric to D4Z4: adenine nucleotide translocator gene (ANT1), actin associated LIM protein (ALP), factor eleven (FXI), plasma prekallikrein (KLK3), and FSHD related gene 1 (FRG1), using muscle tissue from normal control individuals, and from FSHD patients. To date, we have examined expression of ALP and ANT1 in ten unrelated FSHD patient muscle biopsies, and ten normal control muscle samples. We have found a three fold increase in expression of ALP in patients ($p=0.006$) and a four fold increase in expression in ANT1 ($p=0.001$), with the competitive RTPCR approach. Using RTPCR with comparison to housekeeping genes, we have found an increase in expression of FRG1 in four unrelated FSHD patients compared to four unrelated normal controls. Expression levels of KLK3 and FXI have not been quantitated, because they are not consistently expressed in muscle tissue. KLK3 was expressed in four of ten FSHD muscle samples and one of nine normal muscle samples, while FXI was expressed in five of eight FSHD muscle samples and three of seven normal control samples. These results suggest that deletions of the D4Z4 repeats affect expression of genes lying closest to the D4Z4 locus. We are continuing our evaluation of expression at 4q35 by looking at expression levels of these genes in non-FSHD disease muscle controls, as well as investigating expression of non-4q35 housekeeping genes in FSHD muscle and normal control muscle. Supported in part by grants from the FSH Society, MDA-USA,and New York State Dept. of Education.

Program Nr: 1655 from the 1999 ASHG Annual Meeting

***CRX* mutation analysis in age-related macular degeneration (AMD).** *C.L. Freund[1], A. Huang[1], Y. Liu[2], B. Muskat[1], S.G. Jacobson[3], J. Seddon[4], D.J. Zack[2], R.R. McInnes[1]*. 1) Hospital for Sick Children, Toronto, ON; 2) Johns Hopkins Medical School, Baltimore, MD; 3) University of Pennsylvania, Philadelphia, PA; 4) Massachusetts Eye and Ear Infirmary, Boston, MA.

The CRX homeodomain protein is expressed specifically in photoreceptors and pinealocytes, and activates the transcription of photoreceptor outer segment genes, including rhodopsin. Mutations in *CRX* are associated with cone rod dystrophy, and other retinal degeneration phenotypes that affect photoreceptor cells. These findings led to the hypothesis that *CRX* mutations may cause AMD, a photoreceptor degeneration with complex genetics and environmental risk factors. We screened the *CRX* gene in 214 AMD patients (156 atrophic form, 58 neovascular form). One patient with atrophic AMD was found to be heterozygous for a putative pathogenic mutation, P184L. We also identified three benign sequence variants (S34S, S199S, G209G) in other patients. The P184L change (CCG->CTG) is not polymorphic; it is absent from 1018 other *CRX* genes (62 normals, 447 retinal disease patients). Pro 184 is embedded in a '-gly-pro-ser-' context, which is predicted to cause a 180^{o} hairpin turn in the CRX polypeptide. Substitution of this Pro by Leu is predicted to remove the hairpin and create a hydrophobic patch which may promote aggregation of CRX monomers. Initial biochemical studies of the P184L-CRX protein suggest that this allele leads to a loss-of-function: i) in transient transfection studies with a rhodpsin reporter construct P184L-CRX has reduced transactivation activity; ii) the ability of the P184L-CRX mutant to associate with CRX-interacting proteins in yeast two hybrid assays is reduced two to nine fold. These results indicate that i) *CRX* mutations may be a rare cause of AMD, ii) the diminished transcriptional activity and protein:protein interactions of P184L-CRX result from loss of its secondary structure, and iii) demonstration of the pathogenicity of P184L-CRX would confirm that failure to maintain the photoreceptor outer segment can lead to AMD.

Program Nr: 1656 from the 1999 ASHG Annual Meeting

A new locus for late-onset, progressive, hereditary hearing loss (DFNA20) maps to 17q25. *K.H. Friderici[1], R.J. Morell[2], S. Wei[1], J.L. Elfenbein[1], S. Levine[1], T.B. Friedman[2], R.A. Fisher[1].* 1) Depts of Microbiology, Pediatrics and Human Development and Audiology and Speech Sciences, Michigan State Univ, East Lansing, MI; 2) Laboratory of Molecular Genetics, NIDCD, NIH, Rockville, MD 20850.

Progressive hearing loss is a significant problem in the aging population. By age 80 nearly 50% of individuals have hearing loss that impairs their ability to communicate easily. Late-onset hearing loss can be caused by genes or environmental factors such as exposure to noise or otoxic drugs, or by the interaction of genes and environment. The proportion of late-onset hearing loss that is attributable to heredity is unknown. Here we report the localization of DFNA20, a gene causing dominant, non-syndromic, progressive hearing loss in a 3-generation Midwestern family. Affected family members show a bilateral, sloping, progressive, sensorineural hearing loss, first evident at 6,000 and 8,000 Hz, that can be identified in some family members in the early teens and is clearly evident by the early twenties. As age increases, the degree of hearing loss increases with threshold shifts seen at all frequencies. A sloping configuration is maintained. No dysmorphology is observed and vestibular function is normal by neurological exam. After linkage to known dominant and recessive loci was excluded, a genome-wide screen was initiated using the Weber version 8A marker panel. Positive linkage to markers at 17q25 was found with a maximum two point LOD score of 6.62 to marker D17S784 (q = 0). Informative recombinants refined the DFNA20 critical region to 12 cM between D17S1806 and D17S668. Several candidate genes expressed in the ear and reported to map to the region were considered and eliminated. In summary, the DFNA20 locus was identified and mapped 17q25 in an interval of less than 133.7 cR10,000 on the RH map, which corresponds to ~ 2.5 to 3.3 megabases, assuming 1 cR10,000 ~ 25 kbp. Since the progression of early hearing loss in the family resembles that which is associated with old age in the general population (presbycusis), the identification of the DFNA20 gene may provide insight into the etiology of presbycusis and suggest strategies to ameliorate progressive hearing loss.

Program Nr: 1657 from the 1999 ASHG Annual Meeting

D1152H: A common CFTR mutation associated with highly variable disease expression. *K.J. Friedman[1], W.E. Highsmith, Jr.[3], Z. Zhou[1], P.G. Noone[2], A. Spock[4], J.A. Cohn[5], L.M. Silverman[1], M.R. Knowles[2].* 1) Dep't of Pathology & Lab Medicine, Univ North Carolina, Chapel Hill, NC; 2) Dep't of Medicine, Univ North Carolina, Chapel Hill, NC; 3) Dep't of Pathology, Univ Maryland, Baltimore; 4) Dep't of Pediatrics, Duke Univ, Durham, NC; 5) Dep't of Medicine, Duke Univ, Durham, NC.

D1152H is a CFTR missense mutation associated with reduced chloride currents upon heterologous expression. Commonly associated with isolated CBAVD, we report the D1152H/G542X genotype in three siblings with atypically mild CF surviving into the 7th and 8th decade. One developed a cough and "bronchitis" with mucoid P. aeruginosa infection in early adolescence. Although her sweat chlorides were normal (27 mEq/L), she showed reduced chloride conductance across her nasal epithelium (Cl- diffusion PD, +2 mV). She died of respiratory failure at 65 yrs. Her two brothers had atypically mild disease for years, marked by cough, "bronchitis", "asthma" and/or sinusitis. Both grew mucoid P. aeruginosa in their sputum, had normal sweat chlorides (15 and 29 mEq/L), and one had reduced chloride conductance similar to his sister (+1 mV). Neither brother had fathered children, supported by abnormal semen analysis in one. The oldest brother had an episode of acute pancreatitis at 64 yrs. Each has survived into their 70's despite histories of heavy smoking. Subsequently we have identified a further 17 patients (11 families) with D1152H. Six are male (avg. age ~44 yrs) and eleven are female (avg. age ~30 yrs). Presenting symptoms are variable, although most have normal sweat chlorides. Bronchitis, bronchiectasis, hemoptysis and nasal polyps are typical pulmonary manifestations and respiratory infections include P. aeruginosa, S. aureus, and M. avium. All but one are pancreatic sufficient and four have pancreatitis. Ongoing investigations of these individuals are characterizing their disease and quantitating the epithelial ion transport dysfunction associated with this mutation. In summary, D1152H is associated not only with CBAVD, but also with mild, late onset lung disease and pancreatitis in conjunction with normal sweat chlorides, in this family & others.

Program Nr: 1658 from the 1999 ASHG Annual Meeting

Investigating the underlying genetic causes of idiopathic male infertility in an Irish population. *A.M. Friel[1,2,3], M. Maher[2], M. Glennon[2], A. Nolan[3], J.A. Houghton[1]*. 1) Microbiology Department, National University of Ireland, Galway, Ireland; 2) National Diagnostics Centre, BioResearch Ireland, National University of Ireland, Galway, Ireland; 3) Fertility Unit, University College Hospital, Galway, Ireland.

Purpose: Infertility is a common health problem affecting 15-20% of all couples of reproductive age. Men who are infertile account for about half of these cases. Genetic abnormalities are now thought to account for many cases of idiopathic male infertility. Three different approaches are currently in use here to study the underlying genetic causes of male infertility.

Methods: 1. Several genes on the long arm of the Y chromosome contribute to spermatogenesis, and microdeletions in this region have been associated with infertility. A Polymerase Chain Reaction-based approach has been set up to screen the Azoospermia Factor region of the Y chromosome (AZF region) for the presence of microdeletions. To date, 50 azoospermic/oligozoospermic men have been screened using 18 primers which span the AZF region. The aim of this study is to establish the frequency of microdeletions in an Irish male population, and also to assess the potential fertility of male offspring born using current IVF treatments. **2.** Reverse transcriptase-PCR and Northern blots are being used in this study to determine whether infertile men with an intact AZF region are expressing the candidate genes at the expected level. **3.** Mitochondrial DNA deletions in spermatozoa have been associated with male reproductive disorders. A 'Pure Sperm' gradient/Long Range PCR technique has been developed here to investigate if mitochondrial DNA deletions are associated with the decline of motility in spermatozoa.

Results: No deletions have been detected in our 50 patients. Further primers are now being used to expand the screening area. A 4-5 kb region of mitochondrial DNA has been found deleted in some fractions in 8/11 patient sperm samples. This region is currently being cloned and sequenced to see if it correlates with the previously described 'common' 4977 bp deletion. A normal control study is also in progress here.

Program Nr: 1659 from the 1999 ASHG Annual Meeting

Disruption of mouse SMN exon 7 results in early embryonic lethality. *T. Frugier, F. Tiziano, P. Miniou, N. Roblot, A. Dierich, M. Le Meur, J. Melki.* IGBMC, BP 163 Illkirch (Strasbourg) France.

Spinal muscular atrophy (SMA) is an autosomal recessive disorder characterized by degeneration of lower motor neurons and caused by SMN gene mutations. In order to elucidate the pathophysiology underlying SMA, a conditional mutagenesis of the mouse SMN gene has been undertaken using the Cre-LoxP system. We inserted two loxP sites flanking SMN exon 7 (SMNF7) through homologous recombination in order to delete SMN exon 7, the most frequent mutation found in SMA patients. Heterozygous SMNF7 mice were intercrossed and (SMNF7/F7) mice appear similar in size and morphology to wild type mice. In order to delete SMN exon 7 in all cell types, (SMNF7) mice were crossed with a transgenic line expressing Cre recombinase under the control of CMV promoter (CMV-Cre). Offspring carrying both the SMNF7 allele and CMV-Cre transgene were selected. In the progeny, heterozygous deletion of SMN exon 7 (SMND7/+) was detected in one to two offspring lacking CMV-Cre transgene. (SMND7/+) mice remained indistinguishable from wild type littermate to date (12 months). To validate the construct, RT-PCR amplification of RNA extracted from various tissues of mice carrying SMND7, SMNF7 or wild type alleles was performed. In addition to the full length transcript, a shorter product was generated in (SMND7/+) mice. Sequence analysis revealed transcript lacking exon 7, resulting in a putative protein with a different C-terminal end. (SMND7/+) mice were intercrossed but no live (SMND7/D7) mice were produced among 50 newborns. To determine the timing of the (SMND7/D7) lethality, embryos were analyzed during gestation. At 9.5 d.p.c., a total of 53 embryos were analyzed and 13 of them were shown to be completely resorbed. All resorbed embryos were homozygous for the SMND7 mutation, indicating that homozygous deletion of SMN exon 7 in all cell types results in embryonic lethality during early development. To overcome the problem of embryonic lethality, we generated trangenic line expressing Cre recombinase under the control of neuronal promoter in order to induce SMN exon 7 deletion restricted to neurons, the target cells in SMA.

Program Nr: 1660 from the 1999 ASHG Annual Meeting

Clinical characterization of DFNA15 genetic hearing loss caused by a mutation in the POU4F3 transcription factor. *M. Frydman[1,2], S. Vreugde[2], B.I. Nageris[3], S. Weiss[2], K.B. Avraham[2]*. 1) Genetics Institute, Haim Sheba Medical Center, Tel Hashomer, Israel; 2) Department of Human Genetics and Molecular Medicine, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel; 3) Department of Otolaryngology-Head and Neck Surgery and Department of Audiology, Rabin Medical Center, Petah Tiqwa, Israel.

The genetic heterogeneity of inherited deafness has made it difficult to make correlations between clinical symptoms and particular loci, though with the recent cloning of several genes for non-syndromic hearing loss, genotype-phenotype correlations are now possible. The locus DFNA15 defines autosomal dominant progressive hearing loss in a large extended family from Israel, Family H. Sensorineural hearing loss is associated with a mutation in the transcription factor POU4F3, in which case it is fully penetrant. We studied the audiological parameters of Family H members harboring the POU4F3 8-bp deletion. Hearing was measured by pure-tone audiometry and speech audiometry on all participating relatives of Family H. Immittance testing (tympanometry and acoustic reflexes), auditory brainstem response (ABR), and otoacoustic emissions were done in a selected patient population. Audiological characteristics, such as configuration of audiogram (low, mid, high frequency), shape of slope, severity, and age of onset, were found to be variable. Our results indicate that POU4F3 mutation-associated deafness cannot be identified strictly through clinical evaluation, but only through molecular analysis. Intrafamilial variability suggests that other genetic or environmental factors may modify the age of onset and rate of progression.

Program Nr: 1661 from the 1999 ASHG Annual Meeting

Identification of 4 novel mutations in the CFTR gene, including one de novo mutation. *H. Frye-Boukhriss, K. Rommel, T. Doerk, M. Stuhrmann.* Inst Humangenetik, Med Hochschule Hannover, Hannover, Germany.

By June 1999, more than 800 mutations have been identified in the CFTR gene. Some of these mutations are reaching high frequencies, while most mutations are found in very few families or in even one family, only. We assumed that some of the latter mutations may have arisen de novo. Only 4 de novo CFTR mutations have been reported to date, all of which were novel. To identify novel mutations in the CFTR gene, we performed SSCP and heteroduplex-analysis on DNA samples from 100 patients with typical or atypical CF, who carried only one or none of 20 different CFTR mutations, which we screened routinely for CF diagnostics. Among several known mutations and novel base substitutions, we identified 3 novel frameshift mutations and one complex deletion/insertion: 836delT (exon 6a), 1212delA (exon 7), 1366delG (exon 9) and 1492del24ins9 (exon 9). In accordance with their genotypes, three of the compound heterozygous patients (836delT/DF508, 1366delG/G542X, 1492del24ins9/DF508) were affected with typical CF and inherited the novel mutation from one of their parents and the already known mutation from the other. One patient, however, who was heterozygous for both mutations DF508 and 1212delA, presented with very mild pulmonary but severe gastrointestinal symptoms. Testing of the parents of this 5 years old female patient revealed presence of DF508 in the mother but absence of 1212delA in both parents. Paternity testing with 13 different microsatellites was performed after informed consent and false paternity was excluded. We conclude that in case of a novel mutation in the CFTR gene, both parents should be investigated to determine whether this mutation is de novo, which will result in a decreased recurrence risk for CF in further offspring.

Program Nr: 1662 from the 1999 ASHG Annual Meeting

Overexpression of glutathione peroxidase has no effect on ALS-like phenotype of mutant SOD1 transgenic mice.
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Mutations in SOD1 gene are associated with about 20% of familial amytrophic lateral sclerosis (FALS). Transgenic mice expressing FALS-linked mutations develop FALS-like phenotype. The mechanisms by which mutations in SOD1 gene cause FALS are not known. Some lines of evidence support that increased levels of free radicals or reactive oxygen species are involved in the pathogenesis. For example, dietary supplementation with vitamin E delays onset of clinical disease and slows progression in the mutant SOD1 transgenic mice although it does not prolong survival; it has also been reported that glutathione peroxidase (PE) activity is significantly reduced in precentral gyrus (a region affected in ALS) in sporadic ALS. Glutathione peroxidase is an antioxidant enzyme that scavenges hydroperoxides by using GSH as an electron donor. To test whether PE has a protective role in ALS and to explore if this enzyme can be used as a therapeutic agent, we cross-bred the transgenic mice overexpressing PE and mutant SOD1 transgenic mice. Even though the PE activity was 4-fold higher in brain in PE transgenic mice, the phenotype of the double transgenic mice that overexpress both PE and mutant SOD1 is not significantly different from mutant SOD1-alone mice. These results indicate that PE has no effect on ALS-phenotype of SOD1 transgenic mice, suggesting that hydroperoxides is not involved in the pathogenesis.

Program Nr: 1663 from the 1999 ASHG Annual Meeting

A point mutation in exon 1 of the telomeric survival motor neuron (*SMNt*) gene in a child with progressive spinal muscular atrophy. *V.L. Funanage[1,3], M. Scavina[1], S.M. Kumar[1], J. Johnston[1], M. Chopra[1], H.G. Marks[2].* 1) Department of Medical Research, Alfred I. duPont Hospital for Children, Wilmington, DE; 2) Nemours Children's Clinic, Fort Myers, FL; 3) Department of Pediatrics, Thomas Jefferson University, Philadelphia, PA.

Three genes have been identified in the spinal muscular atrophy (SMA) region: survival motor neuron (*SMN*), neuronal apoptosis inhibitory protein (*NAIP*), and *p44*. The *SMN* gene exists in two nearly identical copies: telomeric SMN (*SMNt*) and centromeric SMN (*SMNc*). Absence of the *SMNt* gene has been observed in approximately 95% of SMA patients. Parsons et al. (Am. J. Hum. Genet. **63**: 1712-1723, 1998) have recently described a novel point mutation in exon 1 of the *SMNt* gene in three unrelated SMA patients. We found a similar type of mutation in a nine-year-old black male with a progressive form of SMA. DW was evaluated at age three because of abnormal gait and absent reflexes. EMG and muscle biopsy were neurogenic. DW was diagnosed with SMA type III and was seen in our clinic at age six because of progressive weakness. He is now nine and non-ambulatory. DNA analysis revealed one copy of the *SMNt* gene and at least a single gene copy of *NAIP*. Sequencing of the *SMN* gene in DW demonstrated a point mutation (A2G) in the *SMNt* gene. A second change was a C®T polymorphism in the 5' UTR of the *SMNt* gene. The mother of DW also carried the A2G mutation as well as the C®T polymorphism. This polymorphism was frequent in the black population (6/12 non-related individuals tested), but was not found in 28 individuals of non-African heritage. Our results indicate that a point mutation in exon 1 of the *SMNt* may correlate with a milder phenotype that can be progressive in nature. In patients with a clear clinical picture of motor neuron disease who do not have a *SMNt* deletion, sequencing should be carried out to look for known or novel point mutations. We are currently determining whether the A2G mutation reduces the association of the encoded SMN protein with SIP1 (SMN interacting protein 1).

Program Nr: 1664 from the 1999 ASHG Annual Meeting

Identification of dosage sensitive genes on 22q11 associated with congenital anomaly disorders. *B. Funke, R. Pandita, F. Grimmer, L. Edelmann, N. McCain, E. Spiteri, A. Shanske, R. Goldberg, A. Skoultchi, R. Kucherlapati, T. Van De Water, B. Morrow.* Albert Einstein College of Medicine, Bronx, NY.

Three congenital disorders, cat-eye syndrome (CES), der(22) syndrome and velo-cardio-facial syndrome/DiGeorge syndrome (VCFS/DGS), result from tetrasomy, trisomy and monosomy, respectively, of part of 22q11. Our goal is to determine the molecular basis of these disorders. A 1.5 Mb region on 22q11, containing over 20 genes, is shared among the three syndromes and is conserved on mouse chromosome 16. We hypothesize that this interval contains dosage sensitive genes required for normal embryonic development. To address this hypothesis, we are taking a genetic approach using the mouse as a model organism. Using the cre/loxP system, the syntenic region on mouse chromosome 16 can be deleted. Transgenic mice carrying subsets of genes from the deleted interval can then be used for genetic complementation, thereby identifying genes which are susceptible to decreased dosage (VCFS/DGS). In addition, the genomic transgenics themselves might reveal phenotypes caused by genes that are susceptible to increased dosage (der(22) syndrome and CES). Towards this goal, we have generated 12 lines of transgenic mice for three different human BACs from the distal half of the 1.5 Mb region. Together, they contain 10 genes including *UFD1L*, *TBX1* and *COMT*. All mice are viable and fertile. Preliminary findings show a behavioral anomaly in one of the lines caused by defects in the vestibular system. In most lines, the BACs appear to be intact as determined by STS content analysis. Quantitative Southern hybridization analysis revealed different BAC copy numbers per line which further increases the range of gene dosages that can be analyzed. We found that the human transgenes are expressed in most of the lines by RT-PCR analysis. The spatial expression of most of the human genes mirrored that of their mouse counterparts, indicating that they may be properly regulated. The mice we generated provide a tool to scan a large region on 22q11 for dosage sensitive genes without any a priori knowledge about their function and might help to understand the molecular etiology of VCFS/DGS, der(22) syndrome and CES.

Program Nr: 1665 from the 1999 ASHG Annual Meeting

Differences in complexity of the isolated brachydactyly type c phenotype can not be attributed to locus heterogeneity alone. *R.J.H Galjaard[1], L.I van der Ham[2], N. Posch[2], P.F. Dijkstra[3,4], B.A. Oostra[1], S.E.R. Hovius[2], G.J. Sonneveld[2], A.J.M. Hoogeboom[1], P. Heutink[1]*. 1) Clinical Genetics, University Hospital Dijkzigt, Rotterdam, Netherlands; 2) Plastic and Reconstructive Surgery, University Hospital Dijkzigt, Rotterdam, Netherlands; 3) Radiology, Jan van Breemen Institute, Amsterdam, Netherlands; 4) Radiology, Academic Medical Centre, Amsterdam, Netherlands.

The inherited brachydactyly type C (bdC) phenotype (OMIM 113100), occuring as an isolated malformation, mostly follows an autosomal dominant pattern of inheritance with a marked variability in expression. This phenotype has been mapped to 3 different loci on chromosomes 12p, 12q24, and 20q11.2. The latter locus contains the cartilage derived morphogenetic protein (CDMP)1 gene. A null mutation in this gene has been found in patients with bdC, showing malformations of only the upper extremities. It has been suggested that the gene defect causes anomalies during a very restricted time in embryonic development; the development of the upper limbs are 1-2 days ahead of the lower limbs. Since a more complex bdC phenotype was mapped to chromosome 12q24, it has been hypothesized that the differences in complexity of these phenotypes could be attributed to locus heterogeneity. The editors of OMIM suggest clinical subtyping of the bdC phenotype, based on the degree of complexity. We present two families with patients with bdC, showing considerable intra- and interfamilial variability in expression of the phenotype. We show, using haplotype analysis, that the disease phenotype in both families segregates with markers on chromosome 20q11.2. We found a disease causing mutation in patients of both our families, thereby showing that mutations of the CDMP1 gene can also cause a complex phenotype. We therefore reject both hypotheses mentioned and can not find a justification for clinical subtyping of this phenotype based on the genetic defect. We attribute differences in complexity of the bdC phenotype to variation in expression not due to locus heterogeneity, but to genetic modifiers and/or environmental factors.

Program Nr: 1666 from the 1999 ASHG Annual Meeting

PFGE analysis of 4qter-10qter subtelomeric exchanges in normal and FSHD affected individuals: implications for the molecular diagnosis and genetic counseling of the disease. *G. Galluzzi[1,2], L. Colantoni[2], E. Bonifazi[1], G. Deidda[1], S. Cacurri[1], F. Mangiola[2], E. Ricci[3], L. Felicetti[1]*. 1) Inst of Cell Biology, CNR, Rome, Italy; 2) Centre for Neuromuscular diseases.UILDM-Rome section; 3) Institute of Neurology-Catholic University.Rome.

Interchromosomal exchanges within the subtelomeric regions from 4q and 10q reach high frequency (about 30%) in the normal population and result in the displacement of BlnI-resistant and BlnI-sensitive KpnI repeats from one chromosome to the other, with the appearance of atypical PFGE pattern of p13E-11 alleles.The exchanges may affect the whole set of BlnI-sensitive or BlnI-resistant repeat units or may be only partial: in the latter case hybridization with cloned KpnI repeats is required to identify these types of rearrangement. We tried to verify to what extent interchromosomal exchanges affect the reliability of molecular diagnosis in a group of FSHD families (more than 180) submitted to DNA analysis. In 95% of FSHD patients one of the 4q alleles is small (10 to 30 kb) whereas the other is in the normal range (30 to 200 kb) and both are resistant to BlnI digestion. After PFGE analysis, we observed variation in the number of BlnI-resistant alleles in five patients: 3 trisomic and 1 tetrasomic showed extra-alleles ranging between 35 and 120 kb in size.One patient showed only three EcoRI alleles suggesting a deletion spanning p13E-11 and KpnI sequences and maintained only one 35 kb BlnI-resistant fragment (monosomic). In addition we found three patients showing a pair of small BlnI-resistant fragments, either of similar or different size (22 and 23 kb, 24 and 14 kb, 22 and 19 kb). It is likely that only one of the fragments is involved in the disease, while the other derives from a 10qter chromosome carrying BlnI-resistant repeats. In two of the three cases, family studies allowed us to attribute the pathogenic effect to one of the small fragments. Our results suggest that one must be cautious, at least in a few cases, in the interpretation of molecular data for the diagnosis and genetic counseling of FSHD.

Program Nr: 1667 from the 1999 ASHG Annual Meeting

The human and mouse homologues of the epilepsy gene, EPM2A: Isolation and characterization of full-length transcripts and mutation detection in Lafora disease families. *S. Ganesh[1], K. Shoda[1], K. Amano[1], A. Uchiyama[2], S. Kumada[2], A.V. Delgado-Escueta[3], K. Yamakawa[1]*. 1) Neurogenetics Laboratory, RIKEN Brain Science Institute, Wako-shi, Japan; 2) Tokyo Metropolitan Medical Center for Severely Handicapped, Tokyo, Japan; 3) Comprehensive Epilepsy Program, UCLA School of Medicine, Los Angeles.

The progressive myoclonus epilepsy of the Lafora type (LD) is a rare but lethal autosomal recessive disorder, characterized by the presence of intracellular inclusion bodies. LD usually manifests as epileptic seizures during adolescence followed by a rapid and progressive dementia. Recently, mutations in the novel gene EPM2A, coding for a protein-tyrosine phosphatase, has been identified in a number of LD families but the isolated cDNA was a partial clone and therefore the complete coding region of EPM2A is not known. We have cloned and analyzed the expression of a full-length and an alternatively spliced transcript for the EPM2A gene. Sequence analyses of the full-length clone predicted four exons and is expected to code for 351 amino acids with a protein-tyrosine phosphatase domain. Northern analyses revealed that the size and expression profile of the full-length clone is distinct from the abundantly transcribed transcripts. The alternate transcript is a partial cDNA clone but showed an unique 5 coding sequence suggesting the presence of more than one promoter for EPM2A regulation. We have screened LD families for sequence variations in the coding region of EPM2A and identified polymorphism and mutations, including a microdeletion. In addition, we have cloned and characterized the mouse homologue, named Epm2a, which showed 86% identity at the nucleotide level and 89% identity and 94% similarity at the amino acid level to the human EPM2A. In mouse, Epm2a is located on the proximal end of chromosome 10 and expressed ubiquitously.

Program Nr: 1668 from the 1999 ASHG Annual Meeting

The L392V mutation of Presenilin 1 associated with autosomal dominant early-onset Alzheimers disease alters the secondary structure of the hydrophilic loop. *R. Gantier[1, 2], C. Dumanchin[1], D. Campion[1], C. Loutelier[3], C. Lange[3], J. Gagnon[4], D. Davoust[2], F. Toma[2], T. Frebourg[1]*. 1) INSERM EPI 9906, Faculté de Médecine et de Pharmacie, IFRMP, Rouen; 2) Laboratoire de RMN, UPRES A CNRS 6014, IFRMP, Mont Saint-Aignan; 3) Laboratoire de spectrométrie de masse bioorganique, UPRES A CNRS 6014, IFRMP, Mont Saint-Aignan; 4) Institut de Biologie Structurale, Grenoble, France.

Autosomal dominant early-onset Alzheimers disease results mainly from mutations of the presenilin 1 (PSEN1) gene, which codes for an integral membrane protein of 467 amino-acids. The hydrophilic loop (amino-acids 263-407) of PSEN1, in which many pathogenic mutations have been localized, appears to be crucial for the protein function since it includes the binding domains to different PSEN1 partners. Using circular dichroism (CD), we analyzed the structural effects of the pathogenic L392V mutation that we previously identified in a family including 38 affected subjects, and compared them to those of the E318G substitution. This study revealed that, the L392V mutation, in a phospholipidic medium which mimics the in vivo membrane environment, reduces the a helix content of the PSEN1 loop, whereas the E318G substitution, considered as a polymorphism, does not. These results suggest that the pathogenic effect of some PSEN1 mutations within the hydrophilic loop could be the alteration of the interaction to the different binding proteins through a disruption of the secondary structure.

Program Nr: 1669 from the 1999 ASHG Annual Meeting

***CBP* mutation detection of the Rubinstein-Taybi syndrome for diagnostic purposes.** *R.H. Giles[1], J.G. Dauwerse[1], R.I. Blough[2], A.M. Rus[1], G.J.B. van Ommen[1], D.J.M. Peters[1], M.H. Breuning[1], F. Petrij[1,3]*. 1) Dept Human and Clinical Genetics, Leiden Univ Medical Center, Leiden, The Netherlands; 2) Cincinnati Center for Developmental Disorders, Children's Hospital Medical Center, Cincinnati, OH; 3) Dept Clinical Genetics, Erasmus University, Rotterdam, The Netherlands.

The Rubinstein-Taybi syndrome (RTS) is a malformation syndrome characterized by facial abnormalities, broad thumbs, broad big toes and mental retardation. In a subset of RTS patients, microdeletions, translocations, and inversions involving chromosome band 16p13.3 can be detected. We have previously shown that disruption of the gene encoding the CREB-binding protein (*CREBBP*, or *CBP*), either by these gross chromosomal rearrangements or by point mutations, leads to RTS. CBP is a large nuclear protein involved in transcriptional regulation, chromatin remodeling, and the integration of several different signal transduction pathways. Here we report *CBP* diagnostic analysis in a series of 197 RTS patients. In one case the mother is also suspect for having RTS. Analysis of the entire *CBP* transcript in 37 RTS patients by the protein truncation test revealed 3 (8%) truncating mutations: two point mutations, and one 11 bp deletion. Screening *CBP* for larger deletions, using different cosmid probes in FISH, we found 14/171 (8%) microdeletions. In a systematic and unbiased study of a subset of these patients using 5 cosmid probes covering the entire gene, we found 8/89 (9%) microdeletions. It is of interest to note that 4 of these 8 microdeletions (50%) affected 5' or interstitial sequences of *CBP* and would not have been detected using the commonly used 3' probe RT1. These data demonstrate the necessity of using all 5 probes in FISH screening of RTS patients.

Program Nr: 1670 from the 1999 ASHG Annual Meeting

***G530S* in *COL5A1* is responsible for intrafamilial variability in a three-generation family with the classical type of Ehlers-Danlos syndrome due to a *G1489D* mutation.** *C. Giunta, B. Steinmann.* Dept. Metabolic & Molecular Diseases, University Children's Hospital, Zurich, Switzerland.

The classical type of EDS is an autosomal dominantly inherited connective tissue disorder characterised by skin hyperelasticity, tissue fragility, and joint hypermobility. We investigated the molecular defect of EDS in a three-generation family. Cultured dermal fibroblasts from the proband and his affected daughter produced abnormal a1(V) and a2(V) collagen chains. Mutation analysis revealed the presence of a heterozygous *G1489D* mutation in the *COL5A1* gene, which represents the first report of a glycine substitution in the main triple-helical region of a1(V) collagen. In the proband, the unaffected daughter and mother we identified a further newly recognised *G530S* substitution in the NH_2-terminal domain, which did not co-segregate with the EDS phenotype and was found in only one of 51 unrelated control individuals. The question now arises whether we should consider this a functional mutation or a rare polymorphism. Since the NH_2-terminal domain plays a crucial role in modulating fibril formation, the *G530S* substitution may alter the structure and function of this region and consequently the formation of collagen fibrils. Indeed, indirect evidence supports our hypothesis: (1) the EDS phenotype in the compound heterozygous proband is more severe than in his affected daughter with the *G1489D* mutation only; (2) his unaffected daughter and mother with the *G530S* substitution present with thin skin and delayed wound healing; (3) as did the control individual with the same substitution unlike his mother and sister without *G530S* (the father was not available). Thus, in the compound heterozygous proband the EDS phenotype is caused by the *G1489D* mutation and aggravated by the *G530S* substitution. This represents one of the few examples of intrafamilial variability of heritable disorders of connective tissue caused by a single additional factor. It remains to be studied whether in selected individuals of a large control population or in members of different ethnic groups with somewhat weak connective tissues the *G530S* substitution is more prevalent and predisposes to soft skin.

Program Nr: 1671 from the 1999 ASHG Annual Meeting

Association of specific mutations and severe mental retardation in Duchenne Patients. *R.R Gomes[1], A.F.B. Torres[1], M. Vainzof[1,2], A.M. Cerqueira[1], M.R. Passos-Bueno[1], M. Zatz[1].* 1) Centro de Estudos do Genoma Humano, Departamento de Biologia,IB-USP; 2) Dept. of Neurology, FMUSP, Universidade de Sao Paulo, SP, Brazil.

Duchenne muscular dystrophy (DMD), a lethal X-linked disorder is caused by a defective gene at Xp 21. The DMD gene has 79 exons spanning over 2,4 Mb. About 60-70% of DMD are caused by deletions, 6-8% by duplications and the remaining by point mutations. Several dystrophins isoforms are expressed in different tissues. Dp 140, a 7.5 Kb transcript which encodes a 140 kDa protein is expressed in the CNS and is transcribed from a promoter in the dystrophin locus upstream to exon 45, with its first initiation codon in exon 51; Dp 116 (an intermediate dystrophin gene product of 116 kDa) is expressed in the Schwann cells of the peripheral nerves ; and Dp 71(a protein of 75 kDa expressed in a wide variety of tissues), the major transcript in adult brain arise from a promoter localized within intron 63 of the human DMD gene. About 30-50% of DMD patients have severe to mild MI. It is still unclear whether or how defects in brain dystrophin isoforms are responsible for MI seen in DMD. In order to assess if there is an association between MI in DMD and specific mutations we are currently screening DMD patients with MI for point mutations in the exons involved with the dystrophin brain isoforms, that is: 44,45,49-54,69,70, and 79. Among 143 patients with MI screened for exons 44, 45, 50 and 51 we observed 27 changes: 8 polymorphisms (or rare neutral mutations) and 19 pathogenic mutations. Interestingly, 5 patients with severe MI had novel point mutations in exon 70, which were not observed in 166 patients with normal intelectual capacity. According to Human Gene Mutation Database a total of 216 mutations have been reported in the dystrophin gene. These mutations are distributed along the entire gene with no " hot spots" for specific mutations. Therefore these results suggest that mutations in the exons involved with brain dystrophin isoforms may be more often associated with MR. Supported by FAPESP, CNPq, PRONEX.

Program Nr: 1672 from the 1999 ASHG Annual Meeting

Mutation and polymorphism analysis in the human EPM2 gene in Lafora disease patients. *P. Gomez-Garre[1], Y. Sanz[1], S. Rodriguez de Cordoba[2,3], J.M. Serratosa[1].* 1) Servicio de Neurologia, Fundacion Jimenez Diaz, Madrid, Spain; 2) Unidad de Patologia Molecular, Fundacion Jimenez Diaz, Madrid, Spain; 3) Departamento de Inmunologia, Centro de Investigaciones Biologicas, Consejo Superior de Investigaciones Cientificas, Madrid, Spain.

Progressive myoclonus epilepsy of the Lafora type or Lafora disease (EPM2) is an autosomal recessive disease characterized by epilepsy, myoclonus, dementia, and periodic acid-Schiff-positive intracellular inclusion bodies. We have recently cloned the gene responsible for chromosome 6q-linked Lafora disease (EPM2) and characterized the corresponding product, a putative protein tyrosine phosphatase (LAFPTPase). We now report haplotype and mutational analysis of the EPM2 gene in 74 chromosomes from 37 affected individuals. Ten novel mutations were found: four missense mutations (F84L, R108C, G240K, and P301L), one nonsense (Y86stop), three <50 bp microdeletions (K91fs, Ex1-32bpdel, and Ex1-33bpdel), and two >3 kb deletions affecting exon 2. Including eight mutations previously communicated by our group, to date a total of 18 mutations have been found in our family set. We have also characterized five simple sequence repeats (SSRs) and two simple nucleotide polymorphisms (SNPs) within or adjacent to the EPM2 gene. Haplotypes were constructed and analyzed in order to determine haplotypic associations between EPM2 polymorphisms and EPM2 mutations. R241stop, the predominant mutation in the Spanish population was found in 16 chromosomes. The variability of the haplotypes associated with the R241stop mutation supports the idea that R241stop is a recurrent mutation with several phylogenetic origins. G279S, the second most common mutation, was found in four chromosomes carrying three different haplotypes, a finding that also supports several origins for G279S. All other mutations were found in three or less chromosomes. We conclude that a remarkable genetic allelic heterogeneity is present in chromosome 6q-linked Lafora disease and that even the most common mutations have several phylogenetic origins.

Program Nr: 1673 from the 1999 ASHG Annual Meeting

Wolframin mutations in Spanish families with Wolfram syndrome. *M. Gomez-Zaera[1], T. Strom[2], T. Meitinger[2], V. Nunes[1]*. 1) Molecular Genetics Dept., Institut de Recerca Oncològica, Barcelona, Spain; 2) Abteilung Medizinesche Genetik, Klinikum Innenstadt, München, Germany.

Wolfram syndrome (WS) is an autosomal recessive disorder mainly characterized by familial diabetes mellitus and optic atrophy. WS patients frequently show other clinical features such as diabetes insipidus, renal abnormalities, psychiatric disorders and a variety of neurologic symptoms: deafness, ataxia, peripheral neuropathy. Prevalence reported to date ranges from 1/770,000 to 1/100,000. Our group demonstrated mitochondrial implication in some Wolfram families by linkage analysis taking the presence of mtDNA deletions as a marker. 7 out of 15 families showed deletions (5 multiple deletions, 2 single deletions). A gene mutated in Wolfram patients has been recently located in 4p16. Subsequently named wolframin, this gene encompasses 33.4 kb and it is composed of 8 exons. We have looked for wolframin mutations in 12 of these 15 Wolfram pedigrees. Mutations in 8 families have been detected in exons 4 and 8. Some polymorphisms are also found in exons 2, 3 and 8. A 16 pb-insertion in exon 4 is present in 4 families, thus being the most frequent among the studied families. We could not find mutations in 4 families, and this fact can have at least two explanations: i) mutations are located in wolframin regions not yet analyzed such as exon I or the promoter region; ii) the gene responsible for WS in these families is not the wolframin gene. This option would support the idea of WS as a genetically heterogenic disease. Considering the presence of wolframin mutations and the presence of mtDNA deletions (multiple or single), our families can be classified under 6 categories. We do not yet have an explanation for these various situations. The knowledge of wolframin protein function would clarify how mitochondrial and nuclear genomes contribute to WS.

Program Nr: 1674 from the 1999 ASHG Annual Meeting

Screening of the Pro250Arg mutation in the FGFR3 gene in patients with isolated coronal craniosynostosis. *A. Gonzalez-del Angel, A. Rasmussen, L. Orozco.* Human Genetics Department, Instituto Nacional de Pediatria, Mexico city, Mexico.

Background: The main clinical feature of craniosynostosis syndromes is the premature fusion of one or more of the cranial sutures. It has recently been discovered that several of these syndromes are due to mutations in the Fibroblast Growth Factor Receptor (FGFRs) genes. In the case of isolated coronal craniosynostosis, a number of patients carry the Pro250Arg mutation in the FGFR3 gene which is a substitution of Proline for Arginine at position 250. This condition has been termed Muenke syndrome and has an autosomal dominant inheritance pattern. **Objective**: The purpose of the present study was to determine the presence of the Pro250Arg mutation in the FGFR3 gene in 16 families with isolated coronal craniosynostosis with the aim of delivering an adequate genetic counseling in the positive families. **Methods**: Genomic DNA was obtained from peripheral blood samples of the 16 index cases and their first degree relatives. Exon 7 of the FGFR3 gene was amplified by PCR and the products were cut with the NciI restriction enzyme. **Results**: Of the 16 screened families, only one carried the Pro250Arg mutation. The propositus and his father were heterozygous, with a mutant allele, while his mother and brother were homozygous for the normal allele. The father had been previously considered unaffected, however after a cautious clinical evaluation, he presented only few signs of the disease. After having obtained the molecular diagnosis a risk of recurrence of 50% for future pregnancies was given to this couple who would possibly have been counseled with a much lower recurrence risk otherwise. **Conclusions**: The detection of Pro250Arg mutation in the FGFR3 gene makes it possible to distinguish sporadic cases from those that are familial. In our family with the Pro250Arg mutation there was variable expressivity and its detection showed the importance of the molecular analysis in a family case that previously was not diagnosed by the clinical study.

Program Nr: 1675 from the 1999 ASHG Annual Meeting

Haploinsufficiency for *HOXD8-HOXD13* and *EVX2* causes atypical synpolydactyly. *F.R. Goodman[1], F. Majewski[2], R.M. Winter[1], P.J. Scambler[1]*. 1) Molecular Medicine & Clinical Genetics Units, Institute of Child Health, London, UK; 2) Institute of Human Genetics, University of Düsseldorf, Düsseldorf, Germany.

The critical role of the 5'*HOXD* genes in limb and genital bud development suggests that haploinsufficiency for these genes may cause severe limb and genital malformations. Two children with monodactylous limbs and penoscrotal hypoplasia were indeed recently shown to have chromosome 2q31 deletions that include the entire *HOXD* cluster. In mice, however, hemizygosity for single and multiple 5'*HoxD* genes produces only very minor limb defects. Here we report the phenotype caused by haploinsufficiency for the six most 5' human *HOXD* genes and *EVX2*. Our proband had bilateral 3rd finger duplication, broad halluces, 2nd metatarsal duplication and middle phalanx hypoplasia in both feet; her father had only 3/4 syndactyly in the hands, but an identical foot phenotype. Haplotype analysis with polymorphic markers in and around the *HOXD* cluster revealed that a 2q31 microdeletion had arisen *de novo* in the father and been transmitted to the proband but not to her two unaffected sisters. FISH studies with cosmids mapping to the region, followed by Southern blot analysis, showed that the deletion's centromeric end lay between *EVX2* and *DLX2*, while the telomeric end lay between *HOXD8* and *HOXD4*. These patients are therefore haploinsufficient for *HOXD8-13* and *EVX2*, but for no other gene in the region. Their phenotype closely resembles synpolydactyly (SPD) resulting from expansion or deletion mutations in *HOXD13*, believed to act as dominant negatives and functional nulls respectively. The effects of haploinsufficiency for *HOXD8-12* and *EVX2* thus appear to be slight. An SPD-related malformation also occurs in mice lacking *Hoxd11-13*, but only in homozygotes, implying that the consequences of altered *HOX* gene dosage differ in mice and humans. The limb malformations associated with large 2q31 deletions are therefore due to haploinsufficiency not for the 5'*HOXD* genes, but for a nearby gene(s). Moreover, haploinsufficiency for the nearby gene(s) must mask the SPD caused by haploinsufficiency for the 5'*HOXD* genes, suggesting that the nearby gene(s) interacts with the 5'*HOXD* genes in autopod development.

Program Nr: 1676 from the 1999 ASHG Annual Meeting

The identification of a probable IMPDH pseudogene within the critical region of the X-linked COD-1 locus and theidentification of unique IMPDH-like transcripts within the human retina. *M.B. Gorin[1,2], F.Y.K. Demirci[1,2], B.W. Rigatti[1], N. White[3], J.H. Riley[3], J. Lamb[4]*. 1) Dept. of Ophthalmololgy, The Eye and Ear Institute, Pittsburgh, PA; 2) Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA; 3) Physical Mapping Unit, UK Molecular Genetics, Glaxo Wellcome, England; 4) Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, England.

Inosine 5'-monophosphate dehydrogenase (IMPDH) is the rate-limiting enzyme in de novo guanine nucleotide biosynthesis and is responsible for an X-linked ocular mutant, raspberry in Drosophila. In our search for the gene responsible for X-linked COD-1, we identified sequences from a PAC within our contig that corresponded to the coding region of an IMPDH-like EST . We screened a retina cDNA library (Stratagene) with a hybridization probe that was inclusive of the IMPDH-like sequence from the X-chromosome PAC and which would cross-hybridize with IMPDH-1. Three rare and unique cDNA clones containing inserts of 3936, 951, and 4400 basepairs were isolated. All demonstrated portions of their coding region with strong similarity to IMPDH-1. Fifty-five percent, 57.5%, and 50.1% of the total transcripts for clones 1, 2, and 3, respectively could be closely aligned with human IMPDH-1 with similarities of 90%, 92%, and 88% within the regions of alignment (MacVectors ClustalW algorithm, default parameters). Each of these cDNA clones contained coding regions that were completely unlike the known IMPDH transcripts. We designed specific primers flanking the junctional portions of these cDNAs and confirmed the presence of these unique transcripts in another retina library (Clontech), thus ruling out a simple cloning artifact. Nearly complete sequencing of the PAC has demonstrated the entire coding sequence for an IMPDH transcript without any evidence of introns or homologies to the P2 promoter region of IMPDH-1, strongly suggesting that this is a pseudogene. We are currently identifying the chromosomal locations of these new IMPDH-like genes, testing their expression in other tissues, and confirming that the sequences on the X chromosome represent an IMPDH-like pseudogene.

Program Nr: 1677 from the 1999 ASHG Annual Meeting

Original COFS syndrome Manitoba aboriginal kindred has a mutation in the Cockayne syndrome group B (CSB) gene. *J.M. Graham, Jr.[1], L.B. Meira[2], C.R. Greenberg[3], N.G.J. Jaspers[4], D. Busch[5], D.M. Coleman[5], D.W. Ziffer[5], E.C. Friedberg[2]*. 1) Medical Genetics Birth Defects Cntr, Cedars-Sinai Medical Cntr, Los Angeles, CA; 2) Laboratory of Molecular Pathology, Dept of Pathology, Univ of Texas Southwestern Medical Cntr, Dallas, TX; 3) Children's Hospital, Univ of Manitoba, Winnipeg, Manitoba; 4) Dept Cell Biol & Genetics, Erasmus Univ, Rotterdam, The Netherlands; 5) Dept of Environ and Toxicol Pathol, AFIP, Washington, DC.

Cerebro-Oculo-Facio-Skeletal (COFS) syndrome is a rapidly progressive neurologic disorder leading to brain atrophy with calcifications, cataracts, microcornea, optic atrophy, progressive joint contractures, and growth failure. Cockayne syndrome (CS) is a recessively inherited neurodegenerative disorder characterized by low to normal birth weight, growth failure, brain dysmyelination with calcium deposits, cutaneous photosensitivity, pigmentary retinopathy and/or cataracts, and sensorineural hearing loss. Cultured CS cells are hypersensitive to UV, due to impaired nucleotide excision repair (NER) of UV-induced damage in actively transcribed DNA, whereas global genome NER is unaffected. The abnormalities are caused by mutated CSA or CSB genes. Herein we present evidence that two children related to the Manitoba Aboriginal population group within which COFS syndrome was originally reported by Pena and Shokeir in 1974, have cellular phenotypes that are essentially identical to those previously described for CS. Additionally the identical mutation was detected in the CSB gene from both individuals. Sequence analysis of CSA and CSB cDNAs prepared by RT-PCR failed to identify mutations in the CSA open reading frame. However, in cells from both Manitoba patients we detected the identical deletion of 2 nucleotides at nucleotide position 3794 of the CSB open reading frame. This deletion generates the nonsense codon TGA at codon position 1240 and is therefore expected to result in a truncated polypeptide missing the C-terminal 254 amino acids. Identical heterozygous mutations have been identified in both parents of one of these patients. These results suggest that CS and COFS syndrome share a common pathogenesis.

Program Nr: 1678 from the 1999 ASHG Annual Meeting

Mutations affecting different functional motifs of GLI3 associated with Greig cephalopolysyndactyly syndrome.
K.-H. Grzeschik, M. Kalff-Suske, M. Wessling, D. Bornholdt, H. Engel, H. Heuer. Medical Center for Human Genetics, Philipps-Universitaet, Marburg, Germany.

Greig cephalopolysyndactyly syndrome characterized by craniofacial and limb anomalies (GCPS, MIM 175700), has previously been demonstrated by our group to be associated with translocations as well as point mutations affecting one allele of the zinc finger gene GLI3. Other polydactyly syndromes such as Pallister-Hall syndrome (PHS, MIM 146510) and postaxial polydactyly type A (PAP-A, MIM 174200) are caused by GLI3 mutations. In order to gain more insight into the mutational spectrum associated with a single phenotype, we report here the extension of the GLI3 mutation analysis to more than 24 new GCPS cases. We report the identification of over 15 novel mutations present in one of the patient's GLI3 alleles. The mutations map throughout the coding gene regions affecting different functional motifs. The majority are truncating mutations (9 of 15) that engender prematurely terminated protein products mostly but not exclusively N-terminally to or within the central region encoding the DNA-binding domain. Missense and splicing mutations mapping within the zinc fingers motifs are shown experimentally to interfere with DNA-binding. The five mutations identified within the protein regions C-terminal to the zinc fingers putatively affect additional functional properties of GLI3. In cell transfection experiments using fusions of the DNA-binding domain of yeast GAL4 to different segments of GLI3, transactivating capacity was assigned to two adjacent independent domains (TA1 and TA2) in the C-terminal third of GLI3. Since these are the only functional motifs affected by three C-terminally truncating mutations, we postulate that GCPS may be due either to haploinsufficiency resulting from the complete loss of one gene copy or to functional haploinsufficiency related to compromised properties of this transcription factor such as DNA-binding and transactivation. Comparison of human GLI3 with the homologous Ci (Cubitus interruptus) protein from Drosophila guides to prediction of the functional impact of GLI3 mutations in GCPS which can be tested experimentally.

Program Nr: 1679 from the 1999 ASHG Annual Meeting

Improvement in clinical genetic testing for SCA-7: Development of a Southern to detect extremely large expansions. *P.H. Gunaratne[1], A. Gannavarapu[1], S. Palmer[2], C.S. Richards[1].* 1) Molecular & Human Genetics, Baylor Col Medicine, Houston, TX; 2) Pediatrics, Univ Texas Health Sci Ctr, San Antonio, TX.

Spinocerebellar ataxia type 7 (SCA-7) is a dominantly inherited neurodegenerative disorder involving clinical ataxia due to specific neuronal cell death in the cerebellum. Clinical symptoms include ataxia, ophthalmoplegia, loss of vision, dysarthria, pyramidal and extrapyramidal signs, deep sensory loss or dementia. While a distinguishing feature of SCA-7 from other SCA types is retinal degeneration, the clinical diagnosis is often difficult in the absence of molecular analysis. The expansion of an unstable CAG repeat in the SCA7 gene, which maps to chromosome 3p12-13, is responsible for disease. Normal individuals have 17 or fewer repeats, while SCA-7 patients have expansions of 38 repeats or greater. Anticipation with a prevalence for expansion during paternal transmission has been described in SCA-7 families, and extremely large expansions of over 100 repeats have been reported. While molecular analysis of SCA-7 is available in a number of clinical laboratories (see the GeneTest database), the quality of testing may vary from laboratory to laboratory. The standard clinical laboratory analysis is based upon PCR across the CAG repeat to determine the number of repeats in the normal and expanded alleles. We have now tested over 382 patients for expansions in SCA7. We have identified 37% with two normal-sized alleles, 62% with only one normal-sized allele, and 4% with expansions in the SCA7 gene. However, in patients having only one allele identified by PCR, it is difficult to rule out an extremely large expansion that is not detected by PCR. In addition, primer site polymorphism would also result in failure of a second allele to amplify. To address these concerns, we have developed a Southern-based assay to detect extremely large expansions and thus, rule out possible errors of molecular analysis. We recommend that clinical molecular genetic laboratories providing testing for SCA-7 utilize both PCR and Southern analysis.

Program Nr: 1680 from the 1999 ASHG Annual Meeting

Muscle degeneration without mechanical injury in sarcoglycan-deficiency. *A.A. Hack[1], D.I. Shoturma[2], L. Cordier[2], M.Y. Lam[3], H.L. Sweeney[2], E.M. McNally[3].* 1) Dept. of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL; 2) Dept. of Physiology, University of Pennsylvania School of Medicine, Philadelphia, PA; 3) Dept. of Medicine, Sect. of Cardiology, University of Chicago, Chicago, IL.

In humans, mutations in the genes encoding components of the dystrophin-glycoprotein complex cause muscular dystrophy. Specifically, primary mutations in the genes encoding a-, b-, g-, and d-sarcoglycan have been identified in humans with Limb Girdle Muscular Dystrophy (LGMD) types 2D, E, C and F, respectively. Sarcoglycan is also absent in Duchenne Muscular Dystrophy (DMD) as a secondary consequence of the loss of dystrophin. In order to further study the molecular and physiologic basis of LGMD and DMD we have generated mice deficient for g-sarcoglycan (Hack et al., *J. Cell Biol.* 142:1279). We have shown that mice lacking g-sarcoglycan develop progressive muscular dystrophy similar to human muscular dystrophy. Without g-sarcoglycan, the expression and localization of a-, b- and d-sarcoglycans is compromised. However, the expression and localization of dystrophin, dystroglycan and laminin-a2, a mechanical link between the actin cytoskeleton and the extracellular matrix, appears unaffected by the loss of sarcoglycan. In order to assess the functional integrity of this mechanical link we have subjected sarcoglycan-deficient muscle to isolated muscle mechanics as well as an extended regimen of strenuous exercise. We report that isolated muscles lacking g-sarcoglycan showed normal resistance to mechanical strain induced by eccentric muscle contraction. In addition, sarcoglycan-deficient muscles also showed normal peak isometric and tetanic force generation. Furthermore, there was no evidence for contraction-induced injury in mice lacking g-sarcoglycan that were subjected to an extended, rigorous exercise regimen. These data demonstrate that mechanical weakness and contraction-induced muscle injury are not required for muscle degeneration and the dystrophic process. Thus, a nonmechanical mechanism, perhaps involving some unknown signaling function, is likely responsible for muscular dystrophy where sarcoglycan is deficient.

Program Nr: 1681 from the 1999 ASHG Annual Meeting

Huntingtin interacting protein-1 (HIP-1) associated neuronal death is accompanied by activation of the caspase and JNK pathways. *A.S. Hackam[1], A.S. Yassa[1], J. Vaillancourt[2], M. Metzler[1], L. Gan[1], D.W. Nicholson[2], M.R. Hayden[1]*. 1) CMMT, University of British Columbia, Vancouver, BC; 2) Dept. Biochem. and Molecular Biology, Merck Frosst, Montreal, PQ.

Recent studies on Huntington disease (HD) suggest that events in the cytoplasm occurring prior to aggregate formation and degeneration may be responsible for disease initiation. Mutant huntingtin has significantly reduced interaction with the cytoplasmic protein HIP-1. We have previously demonstrated that HIP-1 promotes cell death and huntingtin aggregate formation in transfected cells. In this study we investigate the molecular basis of HIP-1 toxicity. Transfection of HIP-1 into neuronal NT2 cells resulted in more death than control transfections ($p<0.01$, $n=5$). A cell permeable caspase 3 inhibitor and inhibitors of the stress-kinase JNK both reduced cell death to the level of control, implicating stress kinase and caspase pathways in HIP-1 initiated cell death. A domain within the N-terminus of HIP-1 was identified with homology to proteins known to be involved in apoptosis. Substitution of a conserved hydrophobic residue within this domain with a nonpolar residue (HIP-1/F300G) reduced toxicity to the level of control, whereas substitution to a different hydrophobic residue (HIP-1/F300Y) or alteration of the adjacent amino acid (HIP-1/E299G) did not change toxicity ($n=3$). Also, expression of just this domain alone resulted in toxicity indistinguishable from HIP-1. Our data suggest that HIP-1, unbound to mutant huntingtin, can precipitate neuronal death in HD. Indeed, co-expression of a huntingtin fragment capable of binding HIP-1 significantly reduced cell death, compared with co-expression of HIP-1 with a control protein ($p<0.05$, $n=6$). Therefore, HIP-1 toxicity is likely mediated by the N-terminal domain through activation of caspases and JNK, suggesting that interfering with signaling pathways initiated by excess HIP-1 may be an effective therapeutic strategy.

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Program Nr: 1682 from the 1999 ASHG Annual Meeting

A transcript map of the ALS2 candidate locus on human chromosome 2q33-q34, and exclusion of usurpin, caspase-10, and caspase-8 as candidate genes for ALS2.*ALS2*. *S. Hadano[1,2,3], N. Nichol[3], K. Fichter[3], J. Huizenga[4], J. Skaug[4], J. Nasir[3], D. Martindale[5], B.F. Koop[5], D.W. Nicholson[6], S.W. Scherer[4], G.A. Rouleau[7], J.-E. Ikeda[1,2], M.R. Hayden[3]*. 1) The Inst. of Med.Sci., Tokai. Univ., Kanagawa, Japan; 2) NeuroGenes, ICORP/JST, Japan; 3) CMMT/Dept. of Med. Genet., Univ. of British Columbia, Vancouver, Canada; 4) Dept. of Genet., The Hospital of Sick Children, Canada; 5) Center for Environ. Health, Dept. of Biol., Univ. of Victoria, Canada; 6) Dept. of Biochem. And Mol. Biol., Merck Frosst Centre for Therap. Res., Canada; 7) Centre for Res. In Neurosci., McGill Univ., Canada.

The autosomal recessive form of juvenile amyotrophic lateral sclerosis (ALS2) has previously been mapped to the 1.7 cM interval flanked by D2S116 and D2S2237 on human chromosome 2q33-q34. Based on our yeast artificial chromosome (YAC) contig map (Hadano et al. Genomics 55, 106-112, 1999), this interval spans approximately 3 Mb region of the ALS2 candidate region. To understand the molecular mechanism for the pathogenesis of ALS2, we have sought to generate a transcript map of the ALS2 locus as a first step of the identification of of the ALS2 gene. So far, we have mapped 67 transcribed DNA sequences including 9 known genes (hORC2L, usurpin, caspase-10, caspase-8, Sentrin, CTLA-4, CD28, ArgBP1, and BMPR2), 3 novel full-length transcripts (CALS-C, CALS-7, and CALS-21) and 55 expressed sequenced tags (ESTs) within this interval. In this report, we further focused on mutation search in three known genes, usurpin, caspase-10, and caspase-8. First, we have defined structures of exon/intron organizations of these genes. The human usurpin, caspase-10, and caspase-8 genes were found to contain 14, 11, and 13 exons, respectively. Then, we have designed primer sets that allow to amplify each exon and exon/intron boundaries, amplified DNA from patients with ALS2 and normal controls, determined each DNA sequence, and compared DNA sequences between patients and normal controls. As a result, no mutation has been found in all exons analyzed. These studies indicate that neither usurpin, caspase-10, nor caspase-8 gene has mutation and/or sequence alterations that associated with ALS2, excluding usurpin, caspase-10 and caspase-8 genes as candidates for ALS2 gene.

Program Nr: 1683 from the 1999 ASHG Annual Meeting

Correlation between genotype and phenotype in Korean patients with spinal muscular atrophy. *S.H. Hahn, S.Y. Won, E.H. Lee, K.N. Cho, S.H. Kim, C.H. Hong.* Dept Pediatrics, Ajou Univ Col Medicine, Suwon, Kyunggi do, Korea.

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder with a large phenotypic variability. The disease can be classified into three clinical types based on age of onset and degree of severity. The genes associated with SMA were mapped to chromosome 5q13 where large scale deletion have been reported encompassing several genes, the p44 gene, neuronal apoptosis inhibitory protein (NAIP) gene and the survival mortor neuron (SMN) gene. 90% of patients with type 1 have defects in exon7 and 8 of telomeric survival motor neuron (SMNtel) gene, while 67% and 45% of type I patients have defects of NAIP and p44 gene, respectively. So far, no strict correlation between genotype and phenotype has been established. We have screened for the SMN, NAIP and p44 gene deletions in 9 Korean patients with type 1 and 2 with type II. Six of nine type I (66.7%) and one of two type II patients, 10 year-old girl had deletions of above all three genes. The rest of type I and type II patients had the deleted SMN gene only. It appeared that there was no significant correlation between the size of deletion and severity in Korean patients with SMA. In one particular case, the patient was confirmed to have exon 8 of SMNtel gene, but had the deletion of exon 7. The telomeric exon 7 in this patient was converted by centromeric exon 7. We also analysed Ag1-CA and C212 microsatellite markers in order to find the relationship between copy numbers of marker and severity in SMA patients but no significant relationship was revealed. In screening of one hundred normal individuals, two of them showed deletion of SMNcen gene suggesting the frequent rearrangements in the region. Despite the limited number of subjects in this study, gene conversion appears to account for the significant number of Korean patients with SMA and no relationship between genotype and phenotype was suspected. It is conceivable that other unknown factors may play an important role in the pathogenesis of SMA and are also responsible for the severity of the phenotype.

Program Nr: 1684 from the 1999 ASHG Annual Meeting

Highly homologous human anion transporter genes, CLD and DTDST, have partially overlapping expression in colon epithelium. *S.P. Haila[1], P. Höglund[1], U. Saarialho-Kere[2], M.-L. Karjalainen-Lindsberg[3], J. Hästbacka[1], J. Kere[1, 4].* 1) Dept Medical Genetics, Univ Helsinki, Finland; 2) Dept Dermatology, Helsinki Univ Central Hospital, Finland; 3) Dept Pathology, Univ Helsinki, Finland; 4) Finnish Genome Center, Univ Helsinki, Finland.

Congenital chloride diarrhea (CLD) is an autosomal recessive disorder. It manifests as a lifetime watery diarrhea with a high chloride content and is caused by mutations in the DRA/CLD gene. The CLD protein is highly homologous to two other known human anion transporters, namely the proteins encoded by the diastrophic dysplasia sulfate transporter gene (DTDST) and the Pendred syndrome gene (PDS). Mutations in the human DTDST gene cause diastrophic dysplasia (DTD), atelosteogenesis type II, and achondrogenesis type IB. These proteins share common features like their suggested transmembrane topology and function as anion transporters. In vitro functional studies have demonstrated that both CLD and DTDST mediate the transport of sulfate that is inhibited by extracellular chloride. CLD mRNA is expressed in the mature surface epithelium, especially in brush border cells of normal colon. By Northern analysis, DTDST is expressed in a wide variety of human tissues including colon, whereas Northern analysis with the rat dtdst have suggested a more restricted expression profile limited to cartilage and intestine only. Colon expression of DTDST was studied using radioactive mRNA in situ hybridization and compared with that of CLD in colon. The human DTDST gene demonstrated strong colon expression in the top one-third of the crypt and lack of signal both at the surface level and in the bases of the crypts. The localization was partially overlapping with but distinct from that of CLD. Clinical abnormalities known in DTD are restricted to cartilage and bone and no intestinal manifestations are known. In physiological circumstances, CLD has been shown to transport preferentially chloride but it transports also sulfate, whereas the loss of sulfate transport has a major role in the pathogenesis of cartilage growth defects in DTD. Clinical significance of the colon expression of DTDST remains to be evaluated in DTD patients.

Program Nr: 1685 from the 1999 ASHG Annual Meeting

Effects of mutation on Notch function and implications for CADASIL. *T. Haritunians[1], A. Gu[1], C. Hicks[2], J. Boulter[3], G. Weinmaster[2], N.C. Schanen[1].* 1) Human Genetics, U.C.L.A., Los Angeles, CA; 2) Biological Chemistry, U.C.L.A., Los Angeles, CA; 3) Psychiatry and Biobehavioral Sciences, U.C.L.A., Los Angeles, CA.

CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy) is an inherited vascular dementia that occurs in young people lacking risk factors for stroke. Pathologically, there is replacement of the vascular smooth muscle cells of the tunica media of small and medium sized arterioles by an amorphous substance. The loss of integrity of the vessel wall leads to multiple ischemic strokes in the deep white matter, resulting in progressive dementia. CADASIL is caused by missense mutations in the extracellular EGF-like repeat domains of the transmembrane receptor, Notch3. Notch3 is a member of a family of evolutionarily conserved cell surface receptors with known function in cell fate determination during embryonic development. To understand the molecular pathogenesis of CADASIL, we have cloned and sequenced rat Notch3, revealing a 93% and 85% homology to mouse and human Notch3, respectively. Using site-directed mutagenesis, we introduced four point mutations in rat Notch1 and Notch3, which are analogous to those observed in CADASIL patients. Functional characterization of these mutants in transiently-transfected cells allows in vitro analysis of the effect of these mutations on both ligand binding and signal transduction. Cotransfection with CBF1-luciferase reporter plasmids allows scrutiny of the CBF1 dependent pathways that have been shown to be involved in Notch signaling. These investigations provide preliminary evidence for the mechanism of dominance of the mutations (dominant negative versus activating mutations).

Program Nr: 1686 from the 1999 ASHG Annual Meeting

Structural and Functional Analysis of Human Acid Sphingomyelinase: Insights from Niemann-Pick Disease Mutations. *X. He[1], S. Miranda[1], A. Butler[1], E. Mehler[2], M. McGovern[1], E. Schuchman[1].* 1) Dept Human Genetics, Mt Sinai Sch Medicine, New York, NY; 2) Dept Physiology and Biophysics, Mt Sinai Sch Medicine, New York, NY.

Human acid sphingomyelinase (ASM) is the lysosomal enzyme required for the hydrolysis of sphingomyelin to ceramide and phosphocholine. An inherited deficiency of ASM activity leads to Types A and B Niemann-Pick disease (NPD). To investigate the nature of phenotype variation in this disorder, the clinical, biochemical and molecular abnormalities in individual NPD patients was studied. Several new ASM mutations were identified and expressed, but no correlations could be made between phenotypic severity, the location of the ASM mutations within the primary protein sequence, or the residual ASM activity in cultured cells. In the absence of crystallographic data, a 3D molecular model of human ASM was created based in part on the known 3D structures of Saposin and b-Glucuronidase. ASM consists of a saposin domain (residues 82-97) hinging a phosphodiesterase domain (199-464) with a proline-rich domain (182-197). Four Zn+2 binding sites also were identified, and a substrate binding motif (354-388) was predicted based on homologies with lysenin, a sphingomyelin-specific binding protein. Notably, many of the NPD patient mutations occurred at or near the putative zinc or substrate binding sites. His430 also was predicted as an important residue in the ASM active site based on conservation between ASMs from human, mouse and C. elegans, as well as various phospholipase C sequences. The validity of the molecular model was tested by expressing several ASM mutations at sites predicted to be critical for ASM function and demonstrating loss of activity. These results demonstrate that a reliable model of a complex enzyme such as ASM can be created in the absence of crystallographic information and suggests that such models may be useful for predicting the severity of individual disease-causing mutations.

Program Nr: 1687 from the 1999 ASHG Annual Meeting

Dyskerin localises to the nucleolus and mislocalisation is not involved in the pathogenesis of dyskeratosis congenita. *N. Heiss[1], A. Girod[2], R. Salowsky[1], S. Wiemann[1], R. Pepperkok[2], A. Poustka[1]*. 1) Department of Molecular Genome Analysis, Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, Germany; 2) Cell Biophysics, EMBL, Heidelberg, Germany.

The gene responsible for causing the X-linked recessive form of dyskeratosis congenita (DKC, OMIM 305000), DKC1, has been positionally cloned. The early stage of DKC is characterised by nail dystrophy, mucosal leukoplakias, and reticulate skin hyperpigmentation. Patients develop progressive bone marrow failure and this is the major cause of premature death. An increased risk of developing a range of malignancies also exists. To date, 14 different missense mutations, a putative splice site mutation, and a deletion of the last exon, exon 15, have been identified. There are mutation hot spots in the exons 3, 4, and 11. The peptide dyskerin is highly conserved to the rat NAP57, yeast Cbf5p, and Drosophila Nop60B nucleolar proteins which are involved both in the pseudouridylation and in the cleavage of pre-rRNA. We confirmed the nucleolar localisation of dyskerin by microinjecting mammalian cell lines with constructs expressing dyskerin fused to the enhanced green fluorescent protein (EGFP). Staining with the p80coilin antibody showed that EGFP-dyskerin co-localises with the coiled bodies which are functionally linked with the nucleoli. A series of deletion constructs and mutant constructs containing amino acid substitutions introduced by in vitro mutagenesis were generated. This facilitated the delineation of sequences involved in targeting dyskerin to the nucleus and the nucleolus. Further, we demonstrated that EGFP fusions containing mutations detected in patients do not cause dyskerin to mislocalise. In order to quantitatively and qualitatively examine the endogenous localisation of dyskerin in patient cell lines, we are currently characterising monoclonal antibodies. Since a number of functional connections have been made between the nucleolus and aging, it is of interest that some features of DKC have been likened to those of a pre-mature aging syndrome. To address these functional aspects, we are in the process of generating a murine model for the disease.

Program Nr: 1688 from the 1999 ASHG Annual Meeting

Intragenic localisation of Tau mutations correlates with distinct FTD phenotypes. *P. Heutink[1], P. Rizzu[1], S. Rosso[2], M. Joosse[1], R. Ravid[3], W. Kamphorst[4], JC. van Swieten[2].* 1) Dept. Clinical Genetics, Erasmus University, Rotterdam, Netherlands; 2) Dept. Neurology, Erasmus University Rotterdam; 3) Dutch Brain Bank, Amsterdam; 4) Dept.Pathology, Vrije Universiteit, Amsterdam.

Fronto-temporal dementia's (FTD) are a group of presenile dementia's with progressive behavioral changes and often frontal and temporal atrophy. Approximately 40% of cases are familial. Mutations in the microtubule associated protein tau are associated with familial FTD. Patients can roughly be divided in three groups based on localisation of the mutation in the tau gene: 1. Missense mutations within the first two microtubule binding domains. 2. Missense mutations affecting the C-terminus of tau. 3. Mutations increasing the alternative splicing of exon 10. We have compared clinical and pathological characteristics of multiple families with mutations for each of these three groups (6 large Dutch families supplemented with published data). Group 1 show little variation in age at onset and duration of disease. Disinhibition and initiative loss are characteristic. Tau pathology consists of pretangles in neurons and Glia cells consisting mostly of 4-repeat tau isoforms. Group 2 shows a relatively late age at onset and long duration of disease, memory loss early in the disease. Tau pathology consists of pretangles in neurons only, consisting of all 6 tau isoforms. Group 3 Shows large intrafamilial differences in age at onset and duration of disease.Parkinsonian symptoms are prominent. Tau pathology consist of pretangles in neurons and glial cells consisting of 4-repeat tau isoforms. Functional assays of the different mutations in the tau protein offer a partial explanation for the found correlations. Most missense mutation show a (partial) loss of microtubule binding function and an increased capacity for self assembly into insoluble aggregates. Mutations in the C-terminus of tau have a less dramatic effect on microtubule binding and self assembly providing a possible explaining the late onset and duration of disease. Mutations increasing incorporation of exon 10 result in overexpression of 4-repeat tau isoforms consistent with pathology findings.

Program Nr: 1689 from the 1999 ASHG Annual Meeting

Use of Denaturing High Performance Liquid Chromatography (DHPLC) for connexin 26 gene mutation screening. *P. Hilbert, C. Kint, L. Van Maldergem, Y. Gillerot.* Dept Molecular Biol, Inst Pathologie et Genetique, Loverval, Belgium.

Recent progress in the genetics of deafness has shown that mutations in the connexin 26 (GJB2) gene are responsible for a significant proportion of non-syndromic recessive form of deafness. More than 40 mutations have been described but one (30delG) accounts for the majority of those described so far. The carrier frequency of the 30delG mutation varies from 2 % in the USA to 4 % in Italy. In order to estimate the frequency of the different Cx26 mutations in our population (Southern part Belgium), we developped a rapid screening test based on the DHPLC technology. This method based on the automated separation and detection of homo- and heteroduplexes allows the detection of mutations/polymorphisms in a PCR fragment in less than 10 minutes. The optimization of the DHPLC conditions was established on samples with previously sequenced mutations. Interestingly, the 30delG mutation showed a typical pattern. The carriers mutation screening was realised on 367 individuals of whom 35 (9.5 %) showed a modified elution profile and were subsequently sequenced. The 30delG mutation was identified in 16 subjects. It represents a carrier frequency of 4.35 %, one of the highest described so far. For all of them but one (with a second I128I polymorphism) the mutation was already identified from the elution profile before sequencing. Known polymorphisms and mutations were found in 14 other cases: 3 V37I (0.8 %), 9 M34T (2.4 %) , 1 G160S (0.2 %) and 1 R127H (0.2 %). This result confirms the high frequency of the M34T polymorphic variant. Three new variants were detected: V153I corresponding to a G-A transition at position 457 (4 cases - 1 %), G12V due to a G-T transition at position 35 (1 case - 0.2 %) and I128I due to a C to T transition at position 384 (1 case - 0.2 %). If I128I can probably be considered as a polymorphism, the impact of the two other nucleotide changes remains to be elucidated. This study confirms that the DHPLC is a powerful technology for the screening of mutations in connexin 26 gene and allows the rapid identification of the 30delG mutation without the need of sequencing.

Program Nr: 1690 from the 1999 ASHG Annual Meeting

Mutations in non-coding regions of the proteolipid protein (*PLP*) gene in patients with Pelizaeus Merzbacher disease (PMD) and PMD-like disorders. *G.M. Hobson[1], A. Davis[2], N. Stowell[3], E.H. Kolodny[4], V.L. Funanage[1], H.G. Marks[5]*. 1) Department of Research, A I du Pont Hosp for Children, Wilmington, DE; 2) Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA; 3) DuPont Pharmaceuticals Co, Wilmington, DE; 4) School of Medicine, New York University Medical Center, New York, NY; 5) Department of Pediatrics, Nemours Children's Clinic, Fort Myers, FL.

Pelizaeus Merzbacher disease (PMD) is a dysmyelinating disorder of the central nervous system. Duplications or point mutations in exons of the proteolipid protein (*PLP*) gene have been found in most patients with PMD. Direct sequence analysis of the *PLP* gene of 43 patients with either PMD or a clinically similar disorder revealed four mutations in non-coding regions. One patient was normal at birth and developed head oscillations at four months, and ataxia, which has remained stable, at eight months. At two years of age, he has developed spasticity of the lower limbs. A C to T transition was found in this patient at +37 in the 3'UTR of the *PLP* gene. Since an unaffected male member of the family also has the mutation, it is probably a polymorphism. In two brothers with a connatal form of PMD, we found a G to T transversion at IVS6 +3, which caused skipping of exon 6 in RNA prepared from cultured fibroblasts. An eight-year old who developed nystagmus at 16 months and progressive spastic ataxia at 18 months was found to have a 19-base deletion of a G-rich region near the 5' end of intron 3. A very low level of the mutation was detected in peripheral blood lymphocytes of the maternal great-grandmother, indicating that she is probably a somatic mosaic and the mutation arose in her. Another patient had the classical form of PMD. He was found to have a T to C transition at IVS3 +2. This, like the 19-base deletion, is in intron 3, which is involved in *PLP/DM20* alternative splice site selection. These findings indicate that in the evaluation of patients suspected of having PMD or a PMD-like disorder, one needs to look for mutations in introns in addition to looking for duplications or mutations in exons of the *PLP* gene.

Program Nr: 1691 from the 1999 ASHG Annual Meeting

Characterization of H4F5, a candidate modifying gene for spinal muscular atrophy (SMA). *Y. Hofmann[1], F.I. Sieprath[1], C.L Lorson[2], E.J. Androphy[2], B. Wirth[1].* 1) Inst. of Human Genetics, Bonn, Germany; 2) TUFTS-University, Boston, MA, USA.

Spinal muscular atrophy (SMA) is a common autosomal recessive neuromuscular disorder with variable phenotype caused by mutations in the survival motor neuron gene SMN1 and phenotypically modulated by SMN2 copies. Recently, it has been shown that a single nucleotide exchange in SMN2 attenuates the activity of an exonic enhancer and leads to alternative splicing of exon 7 which is the molecular defect that causes SMA. Neighboring genes may also influence the transcription and function of SMN. H4F5, a duplicated gene ($H5F5^T$ and $H4F5^C$), is localized only 6.5 kb upstream from SMN and shows a strong correlation between frequency of deletions and severity of the disease.

Therefore, it has been proposed as a candidate modifying gene for SMA (Scharf et al., Nature Genet, 1998, 20:83-86). To find out whether the two H4F5 copies are identical, we sequenced the coding region of 10 SMA patients who retained only $SMN2/H4F5^C$ and 10 controls who retained only $SMN1/H4F5^T$. No sequence difference was found. Two isoforms of H4F5 ($H4F5_S$ and $H4F5_L$), each containing 3 exons have been reported. The two isoforms vary by the third exon. We identified an additional splicing within exon 3b in both H4F5 copies. Due to a cryptic site, 182 bp of exon 3b were alternatively spliced, producing an additional isoform. Furthermore, we identified a non-transcribed pseudogene of H4F5.

Both $H4F5_S$ and $H4F5_L$ are predicted to form helix-turn-helix proteins, indicating a role in nucleic acid binding. Since snRNPs consist of RNA and are components of the cellular splicing machinery, binding of H4F5 to RNA and thus an effect on SMN splicing as modifyer is of great interest. We have begun the molecular characterization of H4F5 including RNA- and DNA binding, SMN protein association, and affects upon SMN mRNA processing.

Program Nr: 1692 from the 1999 ASHG Annual Meeting

COMP gene mutations result in abnormal collagen fibril morphology. *P. Holden[1], W. Newman[1], C.J.P. Jones[2], M.E. Grant[1], M.D. Briggs[1].* 1) Wellcome Trust Centre for Cell-Matrix Research, School of Biological Sciences; 2) Academic Unit of Obstetrics and Gynaecology, University of Manchester, Manchester, M13 9PT, England.

Cartilage oligomeric matrix protein (COMP) is found in cartilage, tendon and ligament and functions as a large multidomain pentameric glycoprotein. Mutations in COMP result in pseudoachondroplasia (PSACH) and multiple epiphyseal dysplasia (MED), two autosomal dominant skeletal dysplasias characterised by delayed ossification and joint laxity. Electron microscopy of transverse sections of labrum ligament from a patient with PSACH shows irregular collagen fibril morphology; longitudinal sections show abnormal tissue architecture with disruption of collagen fibril orientation, suggesting an important role for COMP in collagen fibril assembly. Using primary cell lines, established from ligament of both affected and normal adults, we are looking at the role of the C-terminal domain of COMP in the development of the extracellular matrix and particularly its role in collagen fibril assembly. In addition, solid phase binding assays using native COMP purified from articular cartilage and recombinant C-terminal COMP expressed in bacterial and mammalian cells, are being used to identify interactions between COMP and various constituent collagens of ligament and cartilage. Cell attachment/spreading assays are also being performed with these proteins. Furthermore, a series of mutant proteins representing the known mutations within the C-terminal domain have been expressed and are currently being studied to determine their effects on the function of this domain. We have shown that native COMP specifically interacts with primary ligament cells and preliminary evidence indicates that C-terminal COMP can interact with type I collagen, suggesting an important role for this protein in the development of the extracellular matrix and subsequent tissue morphology. This work will undoubtedly provide a clearer illustration of the complex matrix of these tissues and lead to a more complete understanding of disease pathology. (This work is supported by the Arthritis Research Campaign and The Wellcome Trust).

Program Nr: 1693 from the 1999 ASHG Annual Meeting

Application of bisulphite genomic modification to determine the methylation status of a 1.2 kb region of neurofibromatosis type 1 (NF1) promoter sequence in benign neurofibromas from 10 unrelated NF1 patients.
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Neurofibromatosis type 1 (NF1) is a common autosomal dominant disorder. The NF1 gene product, neurofibromin, has a GAP related domain (GRD) that is involved in the down regulation of p21ras oncogene activity. The NF1 gene is therefore considered to function as a tumour suppressor gene. Loss of functional activity of tumour suppressor genes is a key factor in tumorigenesis. Inactivating mutations within the coding region of the NF1 gene lead to development of disease expression and tumour formation. An alternative method of tumour suppressor gene inactivation is hypermethylation of CpG dinucleotides within promoter sequences resulting in a significant reduction in protein expression, and is now recognised as an important epimutational mechanism. In practical terms, hypermethylation represents the second hit (somatic) mutation. We have investigated whether such hypermethylation of the NF1 promoter may also be associated with tumorigenesis in NF1-related tumours. The study group comprised DNA samples derived from 10 benign neurofibromas (9 dermal and 1 plexiform), peripheral blood lymphocytes (PBL) DNA from unrelated NF1 patients and suitable control individuals. Molecular analysis of 70 CpG sites encompassed within the 5' end of the NF1 gene sequence was carried out by bisulphite genomic sequencing. Analysis was confined to a 1.2 kb region containing upstream and downstream sequences from the transcription start site (-674 -> +533) which also encompassed the first 50 bases of exon 1. Tumour-associated hypermethylation of 3 separate CpG sites has been identified at position -609 (both alleles in 6 tumours and 1 allele in 2 tumours), at position -406 (1 allele in 3 tumours), and position -383 (1 allele in 2 tumours) upstream from the transcription start site. All other CpG sites remained hypomethylated. These results suggest that hypermethylation of CpG sites within the NF1 promoter is not a common causative factor for benign neurofibroma formation, and indicates that hypermethylation of the NF1 gene promoter is unlikely to be associated with tumorigenesis in these tumours.

Program Nr: 1694 from the 1999 ASHG Annual Meeting

Ataxia with Isolated Vitamin E Deficiencfy: A Novel Mutation in the a-**Tocopherol Transfer Protein Gene.** *W. Hung[1], H. Jiang[1], H.X. Deng[1], S. Wharton[2], T. Siddique[1].* 1) Dept Neurology, Northwestern Univ Medical Sch, Chicago, IL; 2) Seratoga Springs, NY.

Ataxia with isolated vitamin E deficiency (AVED), transmitted as an autosomal recessive trait, is a progressive neurological disease characterized by cerebellar ataxia and symptoms of central and peripheral axonopathy resembling Friedreichs ataxia. The underlying biochemical defect is the failure of a-tocopherol incorporation into very low density lipoprotein (VLDL) in liver cells. a-Tocopherol transfer protein (a-TTP) enhances the transfer of the a-tocopherol form of vitamin E between membrances. The gene for this protein is located on chromosome 8q13. Its mutation has been shown to cause AVED. To date, sixteen different mutations of aTTP have been identified in AVED patients in North Africa, Europe, North America and Japan. We now report a new AVED case with compound mutation in aTTP gene; one mutation occurs in exon 3 as previously reported, which involves a deletion of T at codon 162 and the other mutation involves a substitution of AA with C at codon 72 in exon 2, that leads to a frameshift and premature stop of aTTP synthesis with additional 10 amino acids downstream.

Program Nr: 1695 from the 1999 ASHG Annual Meeting

Nuclear localization, aggregation or ubiquitination of ataxin-2 are not necessary for SCA2 pathogenesis in human or mouse. *D.P. Huynh[1], M.R. Del Bigio[2], K. Figueroa[1], N. Hoang[1], T. Ho[1], S.M. Pulst[1]*. 1) Medicine, Cedars-Sinai Medical Ctr, Los Angeles, CA.90048; 2) Pathology, University of Manitoba, Winipeg, Canada.

Introduction: Instability of CAG DNA trinucleotide repeats is the mutational mechanism of several neurodegenerative diseases resulting in the expansion of a polyglutamine (polyQ) tract. Proteins with long polyQ tracts have an increased tendency to aggregate, often forming intranuclear inclusion bodies. We investigated whether intranuclear aggregate formation or even diffuse intranuclear localization was necessary for SCA2 pathogenesis using cultured cells, human brains, and SCA2 transgenic mouse lines. Methods: To determine the cellular differences between the normal Q22 and the diseased Q58 ataxin-2, full-length SCA2 cDNA containing either 22 or 58 CAG repeats was ligated in-frame into the pEGFPC1 vector, and transiently transfected into COS1 cells. Transfected cells were imaged using a Zeis LSM 310 confocal microscope. To make a transgenic SCA2 construct expressing predominantly in Purkinje neurons, we placed the full-length SCA2 cDNA under the regulation of the Purkinje cell specific promoter (Pcp2). Transgenic and wild type mice were analyzed using a mouse rotarod apparatus to test motor balance and coordination, and immunohistochemical staining using ataxin-2 and calbindin28K antibodies to determine anatomical abnormality caused by the polyQ expansion. Results:In COS1 cells transfected with Q58 or Q22 GFP-ataxin-2 expression constructs and in SCA2 human brains, cytoplasmic but not nuclear aggregates were found. Mice expressing ataxin-2 with Q58 showed progressive functional deficits accompanied by loss of calbindin-28K labeling and subsequent degeneration of Purkinje cell dendritic aborization, but no intranuclear aggregates in Purkinje neurons. Conclusion:These observations suggest that intranuclear localization or ubiquitination is not a shared feature of polyQ diseases, and point to cytoplasmic factors in neurodegeneration. **IntroductionMethodsResultsConclusion**.

Program Nr: 1696 from the 1999 ASHG Annual Meeting

Spinocerebellar ataxia type 8; clinical and molecular studies in Japan. *Y. Ikeda, M. Shizuka, M. Watanabe, K. Okamoto, M. Shoji.* Department of Neurology, Gunma University School of Medicine, 3-39-22 Showa-machi, Maebashi 371-8511, Japan.

Spinocerebellar ataxia type 8 (SCA8) is the first example of hereditary ataxia caused by a dynamic mutation of the untranslated CTG trinucleotide repeat. We examined the clinical and molecular characteristics of Japanese SCA8 patients and also investigated the frequency of the CTG repeat length in Japanese normal elderly controls aged more than 79 years. The numbers of the combined CTA/CTG repeats of six affected SCA8 alleles and normal elderly controls were 106.3 +/- 24.4 ranging from 89 to 155 and 24.3 +/- 4.4 (n=104 alleles) ranging from 15 to 34, respectively. The mean age at onset of our SCA8 cases was 53.8 +/- 19.7 years, with a range from 20 to 73. One father-daughter pair in an SCA8 family showed remarkable clinical and genetic paternal anticipation, which is a rare phenomenon in SCA8. The number increased from father to daughter of the family was +16 CTG repeats with an acceleration of age at onset, resulting in -31 years (from 73 to 42 years). These SCA8 cases chiefly showed symptoms and signs derived from disturbance of the cerebellar system, and the MRI findings demonstrated significant atrophy of the cerebellar vermis and hemispheres. The severity of cerebellar atrophy on MRI was much greater for SCA8 in comparison with SCA6, another example classified as autosomal dominant cerebellar ataxia type III.

Program Nr: 1697 from the 1999 ASHG Annual Meeting

Darier's disease - a dominant skin disorder with a neuropsychiatric component. *N.J.O. Jacobsen[1], I. Lyons[1], B. Hoogendoorn[1], S. Burge[2], P.Y. Kwok[3], M.C. O'Donovan[1], N.J. Craddock[4], M.J. Owen[1].* 1) Psychological Medicine, Neuropsychiatric Genetics, Cardiff, Wales, Great Britain; 2) Department of Dermatology, Churchill Hospital, Oxford OX3 7LJ; 3) Washington University School of Medicine, 660 S. Euclid Ave, Box 8123 St. Louis, MO 63110 USA; 4) Division of Neuroscience, University of Birmingham, Queen Elizabeth Psychiatric Hospital, Birmingham, B15 2QZ.

Darier's disease (DD - OMIM 124200), is a rare, dominantly inherited disorder that affects the skin producing a variety of types of lesion. Close examination of lesional DD skin shows the presence of the abnormal keratinisation (epidermal differentiation) and acantholysis (loss of cohesion) of keratinocytes. A number of clinical studies have described the co-occurrence of various neurological and psychiatric symptoms with DD, including major affective disorder, psychosis, epilepsy, mental retardation, and a slowly progressive encephalopathy. A single locus for DD has been mapped to chromosome 12q23-q24.1 and a variety of missense, nonsense, frameshift, and splicing mutations in the ATP2A2 gene have recently been described in families with DD by Sakuntabhai et al (1999). This gene encodes the sarcoplasmic/endoplasmic reticulum calcium pumping ATPase SERCA2, which has a central role in intracellular calcium signalling. In this study a sample of patients suffering from DD and varying neuropsychiatric phenotypes were analysed for mutations in ATP2A2. The mutations identified were then compared to mutations found in patients with DD only. In neuropsychiatric cases of DD mutations were found to cluster significantly within exons 13-19 of ATP2A2 compared to DD patients where mutations occurred throughout the gene ($p=0.011$), and were found to be predominantly missense mutations (70% versus 35% in DD patients). This suggests that specific mutations in ATP2A2 elicit pleiotropic effects in brain. Subsequent studies of these mutations may provide simple models for complex neuropsychiatric disorders.

Program Nr: 1698 from the 1999 ASHG Annual Meeting

Towards the gene for Lethal Congenital Contracture Syndrome. *N.A. Jarvinen[1,2], P. Makela-Bengs[1], L. Chen[2], K. Vuopala[3,4], L. Peltonen[2]*. 1) 1Department of Human Molecular Genetics, National Public Health Institute, and Department of Medical Genetics, University of Helsinki; 2) Department of Human Genetics, University of California, Los Angeles; 3) Department of Pathology, Lapland Central Hospital, Rovaniemi, Finland; 4) Department of Pathology, University of Oulu, Oulu, Finland.

LCCS is an autosomal recessive syndrome reported so far only in the genetically isolated population of Finland. The incidence in Finland is 1 in 19000. LCCS leads to prenatal death of the fetus before 32 weeks of gestation. The affected fetuses have hypoplastic lungs, hydrops, marked skeletal muscle hypoplasia and contractures of the extremities. The hallmark of the syndrome is the early onset degeneration of the anterior horn motoneurons and the descending tracts, whereas the sensory neurons of the ascending tracts are preserved. We have previously assigned the locus for LCCS to chromosome 9q34.1 by linkage analysis and restricted the region to under 100kb in the vicinity of marker D9 S61 using linkage disequilibrium and monitoring for shared ancestral haplotype. We have built a physical map of BACs with a 10x coverage over the region between markers D9 S904 and D9 S752 which encompasses the marker D9 S61. With the aid of BAC end sequences used as probes for in silico cloning we have located several EST contigs in the critical DNA region. Known interesting positional candidate genes in the region, include Dynamin-1, SPTAN, and glutamine transaminase K. Analyses of these regional candidate genes are ongoing. Sequencing of this region by genome centers at MIT and the Sanger Centre is also close to completion. Identification of the gene defect will form the basis for understanding the pathogenesis of LCCS and will provide novel insight into the normal and disturbed development of motoneurons.

Program Nr: 1699 from the 1999 ASHG Annual Meeting

Molecular genetics of Thalassemia and G6PD Deficiency in Bahrain. *N.A. Jassim[1], S.S. Al Arrayed[1], T. Merghoub[2], R. Krishnamoorthy[2]*. 1) Genetic dep., Salmaniya Medical Centre., Manama, Bahrain; 2) Inserm U458,Jopital R Debre,Paris,France.

Bahrain is an archipelago in the Arabian Gulf with a population estimate of 700,000 inhabitants. Previous phenotype analysis in this country revealed high frequency of Thalassemias ($\alpha$and $\Beta$)and G6PD deficiency, By using a variety of molecular techniques (PCR-RFLP,PCR-DGGE,RDB,differential PCR amplification and DNA sequencing) here we are reporting the precise molecular spectrum of these disorders in the population of Bahrain. For $\Beta$ thalassemia a total of 13 different mutations were identified, of which four mutations (IVSI,3^{1} end (-25bp);Cd39(C$rightarrow$T);IVSI,5(G$rightarrow$C)and IVSII.1(G$rightarrow$A)account for 80%of all βthal alleles.Concerning α thalassemia , five different α thal determinants have been identified. The most common $alpha$ allele is Saudi type poly A signal mutation (AATAAA$rightarrow$AATAAG)followed by the rightward deletion(-$\alpha$$^{3.7}$)and the pentanucleotide deletion(GGTGAGG>GG)in itron1 of α globin gene. Two less frequent alleles (leftward deletion (-$\alpha$$^{4.2}$)and the Turkish type polyA signal mutation (AATAAA>AATGAA) were identified . For G6PD deficiency, our results showed that G6PD Mediterranean variant (nt563C$rightarrow$;188 Ser$rightarrow$Phe) is the most frequent allele in this country. These data allowed us to tailor a cost -effective molecular diagnosis approaches for screening, diagnosis and genetic counselling purposes.

Program Nr: 1700 from the 1999 ASHG Annual Meeting

Mutations in the NKCC2 gene in a large cohort of Bartter's patients. *N. Jeck[1], R. Ruf[2], R. Vargas[3], C. Chauve[5], M. Konrad[1], M. Vollmer[2], L. Guay-Woodford[4], N. Knoers[4], C. Antignac[3], F. Hildebrandt[2], H.W. Seyberth[1], G. Deschênes[5], D. Feldmann[5]*. 1) Dept of Pediatrics, Philips University, Marburg, Germany; 2) Dept of Pediatrics, Freiburg university, Freiburg, Germany; 3) Inserm U423, Hopital Necker, Paris, Frace ê; 4) Dept of Medecine and Pediatrics, Alabama University, Birmingham, USA, Dept of Human genetics, Nijmegen, NL; 5) Dept of Biochemistry and Dept of Nephrology, Hopital Trousseau,Paris, France.

Bartter's syndrome comprises a set of rare inherited tubular disorders. Recent molecular studies have shown that the antenatal form is caused by loss of function mutations in either NKCC2 gene coding for the bumetanide-sensitive Na-K-2Cl cotransporter or in the gene coding for the K channel ROMK1. Furthermore, mutations in the gene encoding the Cl channel CLCKb have been described in patients with a late onset form of Bartter's syndrome called Bartter III or classic Bartter. But, the classification of the Bartter's phenotypes are still in debate and studies on phenotype-genotype correlations need to be performed. We have studied a large cohort of Bartter's patients from different European countries. After previous analysis of ROMK1 and CLCKb genes, 40 families were selected for NKCC2 screening. Molecular analysis were performed by SSCP and sequencing. Fifty percent of the coding region have been analysed. Six new mutations consistent with a loss of function of NKCC2 and four variations are described. Mutations consist of two insertions (1206insT, 1058insTGTG), one nonsens mutation (W192X) and three missens mutations (L196P, C461R, G478R). All the missens mutations involved conserved aminoacids. A frequent polymorphism in exon 10 have been identified (Q484H). This highly informative biallelic intragenic polymorphism can be useful for molecular studies. The different phenotypes corresponding to the new mutations will be discussed.

Program Nr: 1701 from the 1999 ASHG Annual Meeting

Nonsense-Mediated mRNA Decay- useful to the geneticist as well as to the cell? *D. Jeganathan[1], M.F. Fox[1], J.M Young[1], J.R.W Yates[2], J.P Osborne[3], S. Povey[1].* 1) MRC Human Biochemical Genetics Unit, University College London, London, U.K; 2) Department of Medical Genetics, University of Cambridge, Cambridge, U.K; 3) Bath Unit for Research into Paediatrics, Royal United Hospital, Bath, U.K.

Many mutations predicted to truncate the protein product are associated with decreased levels of mutant transcript. This phenomenon, known as nonsense mediated decay (NMD), is an enigmatic surveillance mechanism that may offer the cell some protection against "dominant-negative" nonsense mutations. We explored the implications of NMD in relation to Tuberous Sclerosis (TSC), an autosomal dominant disorder, with mutations in either of two genes, TSC1 or TSC2. Currently, mutation detection requires comprehensive screening of both genes. However, virtually all TSC1 mutations are predicted to truncate the protein product, thus providing a model system to explore NMD. We used polymorphisms in the coding and 3'untranslated region of the gene to investigate imbalance between allelic transcripts in TSC1 patients. This approach allowed us to identify five out of seven TSC1 patients tested blind from a panel of TSC1 and TSC2 patients, with no false positives. Treatment of cells with puromycin, an inhibitor of translation appears to increase the relative amount of mutant message, which may prove useful for RNA-based mutation screening methods. The other aspect to this work has been concerned with addressing the problem of clinical variability in TSC which can even occur within the same family. Here, similar levels of reduction of the mutant transcript were seen in family members and two unrelated patients with the same mutation. We deduce that intra-familial variation is unlikely to result from variabilty in NMD. However, our results do suggest a possible rapid screen to distinguish TSC1 from TSC2 patients by examining frequent polymorphisms in the mRNA. Another intriguing possibility is that a dense single nucleotide polymorphism (SNP) map of transcribed regions might encourage a similar approach to the assessment of candidate genes for diseases whose cause is still unknown.

Program Nr: 1702 from the 1999 ASHG Annual Meeting

Three sisters with XY gonadal dysgenesis and sex reversal - a new variant? *W. Just[1], A. Sinkus[2], L. Andriuskeviciute[2], L. Jurkeniene[2], C. Geerkens[1], I. Reisert[3], W. Vogel[1], A. Baumstark[1]*. 1) Dept Medical Genetics, Univ Ulm, Ulm, Germany; 2) Dept Biology, Kaunas Medical Academy, Kaunas, Lithuania; 3) Dept Anatomy and Cell Biology, Univ Ulm, Ulm, Germany.

Gonadal dysgenesis in females with an XY karyotype may be the consequence of mutations in *SRY*. Since the discovery of *SRY*, mutations in other genes and deletions of defined chromosomal regions have been reported which also may lead to XY sex reversal.

We report on a family with three sisters, all XY females. Histological examination of their gonads showed only ovarian tissues. It consisted of hypoplastic ovarian stroma with degenerated primary and secondary follicles. No testicular structures were apparent. The presence of *SRY* was confirmed by PCR. A sequence analysis of the coding region did not reveal any mutation. Loss of heterozygosity was studied for markers in distal 9p, a region where deletions also contribute to gonadal dysgenesis. From the marker analyses it was apparent that all sisters had two alleles and all inherited the same allele from their father. This may indicate a mutation in a gene (*DMT1*?) from this chromosomal region.

Since the pedigree suggests an X-chromosomal mode of inheritance, we studied the dosage of genes from Xp21 - the DSS region (dosage-sensitive sex reversal). By quantitative Southern blot a double dosage of the *DAX1* gene from this interval was excluded. With a total of 28 microsatellite markers from the X chromosome we screened for co-segregation of the disorder with these markers. They had in common a 15.65 Mb interval ranging from DXS1202 (Xp21.3-p21.2) to DXS1003 (Xp11.3) including the DSS region.

Genetic studies on mutations/deletions of *WT1* and *SOX9* were not considered, because the sisters did not show symptoms of Campomelic dysplasia (*SOX9*) or kidney cancer (*WT1*).

We conclude that mutations rather than dosage differences in a gene(s) from distal 9p or from a region around DSS may be responsible for the XY sex reversal.

Program Nr: 1703 from the 1999 ASHG Annual Meeting

Genetic Analysis of Hearing Loss in Palestinian Kindreds. *M.N. Kanaan[1], H. Shahen[1,2], J. Morrow[3], T. Sobe[2], M.-C. King[3], K.B. Avraham[2], E.D. Lynch[1]*. 1) Department of Life Sciences, Bethlehem University, Bethlehem, Palestine; 2) Department of Human Genetics and Molecular Medicine, Tel Aviv University, Tel Aviv, Isreal; 3) Department of Medicine and Genetics, University of Washington, Seattle WA.

In Palestine, the prevalence of inherited prelingual profound deafness is among the highest in the world, affecting 5-10% of children in some geographic areas. We have initiated a comprehensive study of genetic causes of hearing loss in the Palestinian population. A pilot survey of probands with prelingual bilateral nonsyndromic hearing loss (NSHL) indicates that mutations in connexin 26 (GJB2) account for 30% (8/27) of cases, all of whom were from multiply-affected families. Homozygosity for three connexin 26 mutations was observed: 30delG, 229T to C, and 167delT. One case was a compound heterozygote for 167delT and 233delC. Comparative haplotype analysis of Jewish and Palestinian kindreds indicates that the 167delT mutation probably occurred independently, perhaps multiple times. In four large Palestinian consanguineous kindreds with congenital profound bilateral hearing loss, all known deafness loci (DFNA and DFNB) were excluded by linkage analysis as the cause of inherited hearing loss. Genome wide analysis of linkage is in progress in these four families and should reveal novel hearing-related genes.

Program Nr: 1704 from the 1999 ASHG Annual Meeting

Exclusion of AR-CHED from the chromosome 20 region containing the PPMD and AD-CHED loci. *A.B. Kanis[1], A.A. Al-Rajhi[2], C.M. Taylor[1], W.D. Mathers[1], R. Folberg[1], D.Y. Nishimura[1], V.C. Sheffield[1], E.M. Stone[1]*. 1) University of Iowa, Iowa City, IA; 2) King Khaled Eye Specialist Hospital, Riyadh, Saudi Arabia.

Congenital hereditary endothelial dystrophy (CHED) is a disorder of the corneal endothelium and has been recognized to segregate in families with both autosomal dominant (AD) and autosomal recessive (AR) modes of transmission. AD-CHED has been previously linked to the pericentric region of chromosome 20. Posterior polymorphous dystrophy (PPMD), a corneal endothelial disorder showing phenotypic overlap with CHED has also been previously genetically mapped to this region. The genetic interval containing AD-CHED is within the larger genetic interval containing the PPMD locus. This study sought to determine whether AR-CHED segregating in a consanguineous Saudi pedigree is linked to the previously mapped and overlapping loci for AD-CHED and PPMD on the pericentric region of chromosome 20.

Forty members of a consanguineous Saudi Arabian pedigree segregating AR-CHED were ascertained. Short tandem repeat polymorphic markers from the 20 cM interval on chromosome 20 containing both the PPMD and AD-CHED loci were used to genotype these individuals. LOD score analysis of the genotype data using the MENDEL software package utilizing a model of autosomal recessive inheritance with complete penetrance showed exclusion of CHED from the entire PPMD / AD-CHED interval by utilizing overlapping intervals of LOD scores of at least -2.

The results obtained demonstrate that AR-CHED is not allelic to either AD-CHED, nor to PPMD, although it has been proposed that AD-CHED may be allelic to PPMD. This shows that there are at least 2 genes responsible for CHED and PPMD.

Program Nr: 1705 from the 1999 ASHG Annual Meeting

Small mutations and polymorphisms in the dystrophin coding region in Japanese patients with Duchenne muscular dystrophy. *J. Kawamura[1], T. Ishihara[1], S. Kato[2], K. Yatabe[1], T. Shigeyama[1]*. 1) Dept. Internal Medicine, National Higashisaitama Hospital, Hasuda, Saitama, Japan; 2) Dept. of Microbiology, School of Medicine, Keio University, Tokyo,Japan.

Duchenne muscular dystrophy (DMD) is a severe X-linked lethal myopathy with an incidence of approximately 1 in 3500 male births. The primary biochemical defect in DMD is dystrophin deficiency. The dystrophin gene, which spans approximately 2.4Mb of Xp, has a high mutation rate. About 60% of DMD patients has a deletion mutation and 10% has duplication mutation. Remaining one third of patients with DMD have no gross rearrangements in the dystrophin gene identifiable by Southern blot analysis or multiplex PCR. These Southern-negative cases presumably resulted from small mutations that cannot be detected by current diagnostic screening strategies. We sequenced the entire coding region of dystrophin gene for detection of small mutations in 38 DMD patients without large deletions or duplications by using reverse transcription PCR and direct sequencing, and identified 25 nonsense mutations or small deletions/insertions, 4 intronic mutations and 2 exon skippings, all of which were unique to single patients. There was no clustering of small mutations in contrast to gross deletions and duplications. Whether the abnormality of the dystrophin gene was a small mutation or a gross deletion/duplication containing exons was not associated with specific phenotypes. We also identified 19 different nucleotide substitutions in the coding sequence. The frequency of small mutations in Southern blot-negative patients with DMD in this study was significantly higher than those of previous studies, which may be ascribed to the high efficiency and accuracy of our strategy of using RT-PCR and direct sequencing. Structural analysis of small genetic disorders relevant to DMD by the present method will contribute the molecular pathogenesis of DMD and extend the scope of carrier diagnosis.

Program Nr: 1706 from the 1999 ASHG Annual Meeting

Pathological missense mutations affecting neural cell adhesion molecule L1 disrupt binding to different ligands.

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Neural cell adhesion molecules (CAMs) of the immunoglobulin (Ig) superfamily are multi-domain cell surface glycoproteins that interact with overlapping repertoires of ligands, cell surface partners and signalling molecules. *in vitro* and *in vivo* studies implicate these proteins in a variety of processes during nervous system development including; axon growth, fasciculation, neuronal cell migration and adhesion. We have shown that mutations affecting one of these CAMs, L1, are responsible for the development of an X-linked recessive disorder that involves congenital hydrocephalus, developmental delay, spasticity and adducted thumbs (X-linked hydrocephalus or MASA syndrome). Over 100 different mutations have been described. Almost one half of these alter single amino acids and may highlight important residues for L1, structure, ligand interaction or downstream activity. The vast majority of missense mutations affect the extracellular Ig and fibronecin type III (FNIII)-like domans that are involved in ligand binding. To examine whether these mutations are capable of affecting ligand binding or affect the ability of L1 to reach the cell surface we have used mammalian expression systems to generate mutated L1. Purified, soluble versions of these proteins are used in FACs-based adhesion assays. Using 20 different mutated constructs we have shown that most pathological missense mutations allow cell surface expression but affect L1 binding either to itself or heterophilic ligands F11 and axonin-1. Whilst many mutations disrupt binding to several ligands, a few have selective effects indicating that patient pathology may be due to more than one type of L1 malfunction. Furthermore, these structure:function studies allow us to propose models for L1 homophilic and heterophilic interaction that involve both Ig and FNIII domains. Finally, the morbidity of individual mutations is related to the consequences of the amino acid change for protein structure and ligand binding.

Program Nr: 1707 from the 1999 ASHG Annual Meeting

The congenital chloride diarrhea (CLD) gene is expressed in eccrine sweat gland epithelium. *J.K. Kere[1, 2], S. Haila[1], U. Saarialho-Kere[3], M.-L. Karjalainen-Lindsberg[4], H. Lohi[1], P. Höglund[1]*. 1) Dept Medical Genetics, Univ Helsinki, Finland; 2) Finnish Genome Center, Univ Helsinki, Finland; 3) Dept Dermatology, Helsinki Univ Central Hospital, Finland; 4) Dept Pathology, Univ Helsinki, Finland.

Clinical abnormalities known in CLD are restricted to the intestine and the most prominent clinical feature is a lifelong watery diarrhea rich in chloride. Other tissues are not known to be affected. CLD is caused by mutations in the anion transporter protein encoded by the CLD, or DRA, gene. The CLD protein acts mainly as a Na+ -independent Cl-/OH- exchanger, but it transports also other anions, such as sulfate and oxalate. CLD has been shown to be expressed in the mature surface epithelium, especially in brush border cells of normal ileum and colon. Its expression profile has been shown to be very restricted and limited only to the intestine and seminal vesicle. Characterisation of other tissues and specific cell types that express CLD in vivo is important in elucidating its physiological function.

Immunohistochemistry with the peroxidase-antiperoxidase technique using polyclonal anti-CLD antibodies was performed to study the expression of CLD in normal epithelial tissues from various organs. Among multiple glandular tissues studied, only eccrine sweat glands, in addition to the seminal vesicle, showed CLD protein expression. Abundant immunoreactivity was detected on the luminal side and in intercellular canaliculi of epithelial cells in the coiled secretory part of the eccrine sweat gland epithelium.

Main electrolytes of primary sweat are of sodium, chloride, potassium, and bicarbonate. The mechanisms of electrolyte transportation across cellular borders and to the sweat gland lumen are not clear but complex co-operation of multiple electrolyte transporters have been proposed. Localisation of the CLD protein to the coiled secretory part of the eccrine sweat gland suggests that it may be one of the mediators of electrolyte transportation involved in primary sweat formation.

Program Nr: 1708 from the 1999 ASHG Annual Meeting

Somatic mutations of *PIG-A* in Korean patients with paroxysmal nocturnal hemoglobinuria : Identification of 4 novel mutations. *J.Y. Kim[1,2], S.S. Park[1], Y.W. Chang[1], J.H. Yoon[1], H.R. Moon[2], H.I. Cho[1]*. 1) Dept. of Clinical Pathology, Seoul National University College of Medicine, Seoul, Korea; 2) Green Cross Reference Laboratory, Seoul, Korea.

Paroxysmal nocturnal hemoglobinuria (PNH) is a hematopoietic stem cell disorder due to the somatic mutation of *PIG-A* gene. *PIG-A* mutations result in the formation of clonal blood cells that are deficient in the surface expression of glycophosphatidylinositol (GPI) anchored protein, and those blood cells present various clinical manifestations including hemolysis and thrombosis. Mutation analysis in different countries is an effective tool to elucidate racial or geographical differences, and possible environmental or genetic causes of somatic mutations. We analyzed *PIG-A* in leukocytes of 18 Korean patients with PNH, using dideoxy fingerprinting and sequencing for the exons and their exon-intron boundaries. We found 12 kinds of mutations including four novel ones in 12 cases : 6 deletion (del) or insertions (ins) [del CT at codon (CD) 139, del 22 bp at CDs 165-172, ins A at CD 248, del G at CD 282, del T at CD 316, del 25 bp at CDs 409-417], 6 base substitutions [GGC->GTC at CD 48, TCA->TGA at CD 127, GGA->TGA at CD 302, G->A at 5' splice site in intron 4, G->A at 5' splice site in intron 5, G->A at 3' splice site in intron 5]. The rate of base substitutions (6/12) is almost the same as in Japan (8/16), and more than in Thai (2/14). All but one case (missense GGC->GTC at CD 48) are frameshift or nonsense mutations causing early truncation of protein, and splicing mutation. These findings suggest that absence of GPI is the major cause of PNH rather than abnormal protein.

Program Nr: 1709 from the 1999 ASHG Annual Meeting

Identification of Mutations in CACNL1A3 in Korean Patients with Hypokalemic Periodic Paralysis ; De novo Arg528His mutations. *S.H. Kim[1], K.W Chung[2], S.J. Kim[3], Y. Namkoong[4], D.J. Kim[5], C.C. Lee[1].* 1) Biology, Seoul National Univ., Seoul, Korea; 2) Biology, Kongju National Univ., Kongju, Korea; 3) Biology, Cheju National Univ., Cheju, Korea; 4) Biology, Kangnung National Univ., Kangnung, Korea; 5) Medicine, Samsung Medical Center, Seoul, Korea.

Hypokalemic periodic paralysis (hypoPP) is an autosomal dominant disorder involving the abnormal function of ion channels and is characterized by attacks of paralysis of varying severity, accompanied by a fall in blood potassium levels. Linkage analysis showed that the candidate locus responsible for the hypoPP was localized to chromosome 1q31-32, and this locus encoded the muscle DHP-sensitive calcium channel a1-subunit (CACNL1A3). So far, three different mutations in CACNL1A3 gene were identified in patients with hypoPP: Arg528His, Arg1239His, and Arg1239Gly. In this study, we screened for the CACNL1A3 gene in four Korean families with hypoPP using PCR-based restriction and SSCP analysis. Arg528His mutation was detected in three families and one family has no known mutations. Moreover, for the first time, we detected de novo Arg528His mutations in two out of the three families with hypoPP. Haplotype analysis with three microsatellite makers (D1S1726, CACNL1A3, D1S1723) and CfoI RFLP suggested the evidence of de novo Arg528His mutations in two of three families with Arg528His. These results imply that de novo mutation, in addition to nonpenetrance, is one of the genetic mechanisms which can explain the previous clinical observation that hypoPP occurs sporadically without familial history.

Program Nr: 1710 from the 1999 ASHG Annual Meeting

Novel Mutations of the PKD1 Gene in Korean patients with Autosomal Dominant Polycystic Kidney Disease.

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The gene for the most common form of autosomal dominant polycystic kidney disease (ADPKD), PKD1, has recently been characterized and shown to encode an integral membrane protein, polycystin-1, which is involved in cell-cell and cell-matrix interactions. Until now, approximately 30 mutations of the 3' single copy region of the PKD1 gene have been reported in European and American populations. However, there is no report of mutation in the Korean population. Using the polymerase chain reaction and single-strand conformation polymorphism analysis, 91 Korean patients with ADPKD were screened for mutation in the 3' single copy region of the PKD1 gene. As a result, we have identified and characterized six mutations : three frameshift mutations (11548del8bp, 11674insG, 12722delT), a nonsense mutation (Q4010X), and two missense mutations (R3752W, D3814N). Five mutations except for Q4010X are reported here for the first time. Our findings also indicate that many different mutations are likely to be responsible for ADPKD in the Korean population. The detection of additional disease-causing PKD1 mutations will help in identifying the location of the important functional regions of polycystin-1 and help us to better understand the pathophysiology of ADPKD.

Program Nr: 1711 from the 1999 ASHG Annual Meeting

Analysis of the R402Q polymorphism associated with OCA1B. *R.A. King, J.E. Pietsch, J.P. Fryer, W.S. Oetting.* Department of Medicine, Division of Genetics, Univ of Minnesota, Minneapolis, MN.

Mutations of the tyrosinase gene are responsible for type 1 oculocutaneous albinism (OCA1). The 'classic' form (OCA1A) is associated with lifelong absence of melanin pigment in the eyes, hair and skin, resulting from mutations that produce inactive enzyme. Many individuals with tyrosinase gene mutations have no pigment at birth but eventually develop skin, hair and eye melanin (OCA1B). We and others have shown that this is caused by leaky mutations of the tyrosinase gene that encode enzyme with residual catalytic activity, allowing for some biosynthesis of melanin pigment. One particular tyrosinase gene mutation, an amino acid substitution of arginine by glutamic acid at codon 402 (R402Q) has been reported to be associated with normal enzymatic activity at 31C and markedly reduced activity at 37C. This mutation also represents a common polymorphism with an allele frequency of 85% for the arginine allele (CGA) and 15% for the glutamine allele (CAA). It has been suggested that individuals with the CAA allele on one chromosome and a null activity mutation on the homologous chromosome can also have OCA1B, indicating that this common polymorphism is also pathologic. To analyze this possibility, we have performed haplotype analysis for a large number of OCA1 families utilizing polymorphic changes in the tyrosinase gene at positions 301bp, 199bp, codon 192, and codon 402. We have identified seven families in which an unaffected parent and an affected child have a pathologic mutation on one allele and the CAA allele at codon 402 on the homologous allele. There was no evidence of hypopigmentation or ocular changes of albinism in the parent. We conclude that the R402Q polymorphism is not involved in OCA1.

Program Nr: 1712 from the 1999 ASHG Annual Meeting

Motor and cognitive changes among presymptomatic Huntington disease gene carriers. *S.C. Kirkwood, E. Siemers, M.E. Hodes, P.M. Conneally, J.C. Christian, T. Foroud.* Dept Med & Molec Genetics, Indiana Univ Sch Medicine, Indianapolis, IN.

Although typically diagnosed following the onset of manifest chorea, Huntington disease (HD) is characterized by variable onset of progressive motor, cognitive, and emotional signs. We previously demonstrated subtle differences in several cognitive[1] and motor[2] measures in presymptomatic gene carriers (PSGC) in a subset of the current study sample. In order to further characterize these changes in presymptomatic at-risk individuals, we examined the entire sample of 585 individuals, gene carriers (n=171) and nongene carriers (NGC) (n=414), who were gene tested anonymously and could not be given a clinical diagnosis of HD. A neurologic rating scale, physiological tests measuring speed of movement and reaction time, and the Wechsler Adult Intelligence Scale-Revised (WAIS-R) were administered. On neurologic examination, the PSGC exhibited significantly greater definite or possible abnormality than the NGC for several oculomotor measures, including saccades accuracy, saccades velocity, optokinetic nystagmus, and overall oculomotor function and for functional motor measures that included gait and station stability, rapid alternating movement, and muscle stretch reflexes. Although no gene carriers given a definite diagnosis of HD on clinical examination were included, the PSGC demonstrated more definite or possible abnormality for chorea of the extremities, trunk, and face. PSGC had significantly slower performance for auditory reaction time, visual reaction time with decision, movement time with and without decision, and button-tapping time. Scores on the WAIS-R subtests, Picture Arrangement and Digit Symbol, and Arithmetic were worse for the PSGC. These results suggest that subtle worsening of speed of movement, reaction time, oculomotor, functional motor, and psychomotor performance occurs prior to the onset of signs sufficient to make a clinical diagnosis in HD gene carriers.

1. Foroud T, Siemers E, Kleindorfer D, et al. *Ann Neurol.* 1995;37:657-664.
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Program Nr: 1713 from the 1999 ASHG Annual Meeting

X-linked retinitis pigmentosa 3 (RP3): identification of a retina-specific *RPGR* transcript and gene targeting of the mouse orthologue. *R. Kirschner[1], C. Zeitz[1], K.-P. Knobeloch[2], K. Rüther[3], E. Rohde[2], K. Hoffmann[3], J. Grosse[4], I. Horak[2], R. Roepman[5], T. Rosenberg[6], F.P.M. Cremers[5], H.-H. Ropers[1,5], W. Berger[1]*. 1) Max-Planck-Institut für molekulare Genetik, Berlin, Germany; 2) Forschungsinstitut für molekulare Pharmakologie, Berlin, Germany; 3) Augenklinik der Charité, Berlin, Germany; 4) Anatomisches Institut der Technischen Universitaet Muenchen, Germany; 5) Department of Human Genetics, University of Nijmegen, The Netherlands; 6) National Eye Clinic for the Visually Impaired, Hellerup, Denmark.

Retinitis pigmentosa (RP) is a heterogeneous group of retinal dystrophies characterised by progressive degeneration of the photoreceptor cells. Mutations in the RP3 gene (*RPGR*, retinitis pigmentosa GTPase regulator) were detected in 20% of all X-linked RP families although linkage data suggest that 60% are due to mutations at this locus. To search for additional coding sequences as putative mutation targets we analysed *RPGR* expression in various organs from mouse and man. These studies revealed at least 12 alternatively spliced isoforms. Some of the transcripts are tissue-specific and contain novel exons, which elongate or truncate the previously reported ORF. One of the newly identified exons is exclusively expressed in the human retina and mouse eye and contains a premature stop codon. Moreover, this exon was found to be deleted in a family with XLRP. As alternative splicing often requires regulatory elements in intronic sequences we are now studying adjacent *RPGR* introns by comparative sequence analysis in man and mouse. In order to examine the mechanisms leading to premature photoreceptor cell death we established a mouse model for RP3. Targeted mice harbour an in-frame deletion of exon 4 and show a reduced expression of the *mRpgr* mRNA. Exon 4 codes for 21 amino acids which are identical in man and mouse. Hemizygous male mice carrying the targeted allele are viable and fertile. Up to 4 month of age, no characteristic changes were observed by electroretinography and conventional histology of retina sections. Phenotype analysis of older mice will reveal whether this exon 4 deletion results in photoreceptor degeneration.

Program Nr: 1714 from the 1999 ASHG Annual Meeting

A novel mutation in the troponin I gene is associated with sudden cardiac death in familial hypertrophic cardiomyopathy. *I.C. Klausen[1], J. Mogensen[1,2], H. Egeblad[1], U. Baandrup[4], A.D. Borglum[3]*. 1) Department of Cardiology, Skejby University Hospital, Aarhus, Denmark; 2) Research Unit for Molecular Medicine, Skejby University Hospital, Denmark; 3) Institute of Human Genetics, University of Aarhus, Denmark; 4) Department of Pathology, Aarhus University Hospital, Denmark.

Background: Familial hypertrophic cardiomyopathy (FHC) is inherited as an autosomal dominant disease. Mutations have been identified in 8 different genes all encoding sarcomeric polypeptides. FHC is a major cause of sudden cardiac death among young otherwise healthy individuals. Methods: We made clinical and genetic investigations of a large FHC pedigree. Individuals were considered to have FHC if they had hypertrophy of the myocardium (>13mm), hypertrophy in the ECG or Q-waves/major ST-segment changes in at least 2 ECG leads. Genetic investigations included pairwise and multipoint linkage analyses and mutation analyses of the troponin I gene (TNNI3). Results: Two out of 9 affected family members died suddenly in their fifties. They had both suffered from dyspnoea, angina, and palpitations. The remaining affected family members had symptoms of varying severity due to either dysfunction of the left ventricle or arrhythmia, except a 21 years old asymptomatic woman. The hypertrophy observed was primarily localized at the apex. Linkage analyses of all the known FHC loci appointed TNNI3 as the probable disease gene. Subsequent mutation analyses identified a ser199asn mutation in exon 8. The ser199 is a phylogenetically conserved amino acid, and the nucleotide substitution was not observed in 140 control chromosomes. Conclusion: We describe the first TNNI3 mutation associated with sudden cardiac death in the first Caucasian family ever reported to carry mutations in TNNI3. The mutation appeared highly penetrant and was associated with relatively high morbidity and mortality in the pedigree investigated.

Program Nr: 1715 from the 1999 ASHG Annual Meeting

Cataract due to crystal deposits of 37R>S mutated gamma-D-crystalline. *S. Kmoch[1], B. Asfaw[1], K. Bezouska[3], J. Brynda[4], J. Sedlacek[4], M. Filipec[2], M. Elleder[1].* 1) Inst Inherited Metabolic Dis, Charles Univ 1st Sch Medicine,Prague Prague 2, Czech Republic; 2) Dept Ophthalmology, Charles Univ 1st Sch Medicine, General Faculty Hospital, Prague; 3) Dept Biochemistry, Faculty of Science, Charles University, Prague; 4) Dept of Gene Manipulation, Academy of Science, Prague, Czech Republic.

We found a crystal cataract in an five years old boy suffering from impaired vision. The crystals ranging in size up to 1 mm were mechanically isolated from the extracted lenses, purified and analysed using an SDS-PAGE. Single protein band of molecular mass of 21kDa was subsequently identified as the gamma-D-crystalline (CRGD) using Edman microsequencing. Direct sequencing of CRDG gene showed that the patient is heterozygous for a C>A transversion resulting in a 37R>S substitution in the CRDG protein. The mutation was not found in the mother and two older siblings with normal vision. The father whose vision was reported to be normal, died and no DNA was available for investigation. These findings allow us to conclude that the patient mutation most probably arose de-novo. This is the first case of a human cataract caused by deposition of crystals of CRDG protein and the second case of an cataract associated with mutation of this lens protein. To learn more about the mechanism underlying the crystal formation in this pathological condition, the crystals were subjected to x-ray diffraction analysis. Data have been collected to 2.2A resolution (ESRF, ID 14 EH3). Preliminary results have shown that the substitution of an Arg residue in the Arg37Ser mutant is essential for the observed intermolecular contacts between adjacent crystalline molecules.

Program Nr: 1716 from the 1999 ASHG Annual Meeting

Three novel SALL1 mutations extend the mutational spectrum in Townes-Brocks syndrome. *J. Kohlhase[1], C. Blanck[1], S. Engels[1], P. Burfeind[1], A. Bottani[2], M. Patel[3], W. Engel[1], H.Y. Kroes[4], J.M. Cobben[4].* 1) Institute for Human Genetics, University of Goettingen, Goettingen, Germany; 2) Division of Medical Genetics, University of Geneva, Geneva, Switzerland; 3) Department of Genetics, The Hospital for Sick Children, Toronto, ON, Canada; 4) Department of Medical Genetics, University of Groningen, Groningen, The Netherlands.

Townes-Brocks syndrome (TBS) is an autosomal dominantly inherited malformation syndrome characterized by anal, renal, limb, and ear anomalies. TBS is caused by mutations in the putative zinc finger transcription factor gene SALL1 related to spalt of Drosophila. All mutations identified so far are truncating mutations (nonsense mutations or short deletions), which are located 5' of the first double zinc finger encoding region. This led to the suggestion that only SALL1 mutations which remove all double zinc finger domains result in TBS. Here we present three novel mutations of SALL1, one single base pair deletion, one nonsense mutation, and one intronic mutation. While the short deletion is located within the previously described hotspot region, the nonsense mutation is positioned 3' of the region encoding the first double zinc finger. The intronic mutation creates a aberrant splice site and is predicted to result in a protein in which all but the most carboxyterminal zinc finger domains are intact. Based on these findings, we suggest that all double zinc finger domains of SALL1 are required for SALL1 function, and that truncating mutations removing one or more of these domains from the SALL1 protein result in the same phenotype.

Program Nr: 1717 from the 1999 ASHG Annual Meeting

Novel Mutations and Genotype-Phenotype Relationships in 107 families with Fukuyama-type Congenital Muscular Dystrophy (FCMD). *E. Kondo-Iida[1,3], K. Kobayashi[1], M. Watanabe[1], J. Sasaki[1], Y. Nakamura[2], T. Toda[1].* 1) Lab Genome Med,Hum Genome Ctr, Inst Med Sci, Univ Tokyo , Tokyo, Japan; 2) Lab Molecular Med, Hum Genome Ctr, Inst Med Sci, Univ Tokyo, Tokyo, Japan; 3) Dept Pediatrics, Tokyo Women's Med Univ, School of Med, Tokyo, Japan.

Fukuyama-type congenital muscular dystrophy (FCMD), one of the most common autosomal recessive disorders in the Japanese population, is characterized by congenital muscular dystrophy in combination with cortical dysgenesis. Recently we identified on chromosome 9q31 the gene responsible for FCMD, which encodes a novel 461-amino-acid protein we have termed fukutin. Most FCMD-bearing chromosomes examined to date (87%) have been derived from a single ancestral founder, whose mutation consisted of a 3kb retrotransposal insertion in the 3' non-cording region of the fukutin gene. Two independent point mutations (nonsense and frameshift) in this gene have also been found on chromosomes carrying the non-founder haplotype. FCMD is the first human disease known to be caused primarily by an ancient retrotransposal integration. We undertook a systematic analysis of the FCMD gene in 107 unrelated patients, and identified four novel non-founder mutations in five of them: one missense, one nonsense, one L1 insertion, and a 1-bp insertion. The 1-bp insertion was the first de novo alteration ever confirmed in FCMD. Moreover, we evaluated all the mutations observed so far to look for correlation between genotype and phenotype. The frequency of severe phenotypes, including WWS-like manifestations such as hydrocephalus and microphthalmia, was significantly higher among probands who were compound heterozygotes carrying a point mutation on one allele and the founder mutation on the other, than it was among probands who were homozygous for the 3-kb retrotransposon. Remarkably, we detected no FCMD patients with non-founder (point) mutations on both alleles of the gene, and suggest that such cases might be embryonic-lethal. Our results provided strong evidence that loss of function of fukutin is the major cause of FCMD, and appeared to shed some light on the mechanism responsible for the broad clinical spectrum seen in this disease.

Program Nr: 1718 from the 1999 ASHG Annual Meeting

Mutation analysis of the CFTR and CT genes in patients with idiopathic pancreatitis. *A. Korte[1], J. Ockenga[2], T. Doerk[1], N. Teich[4], M. Ballmann[3], V. Keim[4], M.P. Manns[2], M. Stuhrmann[1]*. 1) Inst Humangenetik; 2) Abt Gastroenterologie und Hepatologie; 3) Kinderklinik, Medizinische Hochschule, Hannover, Germany; 4) Medizinische Klinik II, Universitaet Leipzig, Germany.

Mutations in the cystic fibrosis transmembrane conductance regulatory (CFTR) gene seem to confer susceptibility to chronic pancreatitis in some patients. In addition, autosomal dominant hereditary chronic pancreatitis is frequently caused by mutations in the cationic trypsinogen (CT) gene. We investigated whether mutations in the CFTR or the CT genes are associated with idiopathic non-familial (sporadic) pancreatitis. We screened the CFTR gene from 13 unrelated patients with idiopathic pancreatitis for the presence of 28 mutations and the 5T allele in intron 8 of the CFTR gene. This procedure has been proven to detect > 90% of all CF alleles in the German population. The CT gene was screened by restriction digestion or a mutation specific PCR and additionally by sequencing of exons 1, 2, 3. Patients with a CFTR mutation were further investigated by assessment of the transepithelial nasal potential difference and by intestinal current meassurement. No mutation could be identified in the CT gene. Heterozygosity for the CFTR mutations Y1092X or I336K was present in one male and one female patient, respectively. I336K was present in the healthy mother and sister of the patient, too. With intragenic markers we proved that the sister inherited a different paternal allele. In addition, one female patient carried a 5T allele. Two of these three patients had nasal polyps, but none of them showed any abnormal electrophysiological values. The allele frequency of CFTR mutations is almost twice as high among our patient group (7.7%) than among the general population (4%), while the allele frequency of the 5T allele (3.8%) is not different from the expected frequency (5%). These results are in accordance with two recent studies showing an association between mutations in the CFTR gene and chronic pancreatitis. In contrast to autosomal dominant chronic pancreatitis, mutations in the CT gene do not seem to be a significant factor in the pathogenesis of sporadic pancreatitis.

Program Nr: 1719 from the 1999 ASHG Annual Meeting

DNA binding analysis of PITX2 mutations found in IGDS and ARS patients. *K. Kozlowski[1], M. Walter[1,2]*. 1) Ophthalmology, University of Alberta, Edmonton, Alberta, Canada; 2) Medical Genetics, University of Alberta, Edmonton, Alberta, Canada.

The autosomal-dominant disorders Iridigoniodysgenesis Syndrome (IGDS) and Axenfeld-Rieger Syndrome (ARS) are characterized by maldevelopment of the anterior segment of the eye, associated with an increased risk of early-onset glaucoma. IGDS and ARS both result from mutation of the paired-bicoid class transcription factor PITX2, indicating that they are allelic disorders. We have used an in vitro coupled transcription/translation system to express PITX2 protein. DNA-PITX2 interactions were examined using gel mobility shift assays. Recombinant PITX2 protein was tested in DNA-binding assays with an oligonucleotide containing the CE-3 binding site of Pitx1. Pitx1 and PITX2 have 97% identity within their DNA-binding homeodomains and are thus expected to bind similar DNA sequences. We have demonstrated that wildtype PITX2 can bind the CE-3 oligonucleotide. Mutations identified in IGDS patients (Arg69His; Arg84Trp) were introduced into recombinant PITX2 by site-directed mutagenesis. Preliminary results demonstrate reduced DNA-binding by the mutant proteins, suggesting that diminished DNA-binding by PITX2 is the etiological mechanism of IGDS. We are currently using a mammalian expression vector for PITX2 production to confirm our results, and to investigate the binding ability of ARS-associated mutations of PITX2. As ARS is a more severe phenotype than IGDS, we hypothesize that ARS-mutant PITX2 protein will exhibit an even greater reduction in DNA-binding ability. These experiments will allow correlation between the IGDS and ARS PITX2 mutations and PITX2 protein function, and, hopefully, further elucidate the etiological mechanisms of both disorders.

Program Nr: 1720 from the 1999 ASHG Annual Meeting

DNA microarray-based profiling of global gene expression changes in myotonic dystrophy (DM). *R. Krahe[1], J. Palatini[1], T. Ashizawa[2], K. Virtaneva[1]*. 1) Div. of Human Cancer Genetics, Comp. Cancer Ctr., Ohio State Univ., Columbus, OH; 2) Dept. of Neurology, Baylor Coll. Med. and VA Med. Ctr., Houston, TX.

Myotonic dystrophy (DM) is an autosomal dominant neuromuscular disease with highly variable multisystemic manifestations. The mutation underlying DM is an unstable (CTG)n expansion in the 3' untranslated region of the myotonic dystrophy protein kinase gene (*DMPK*). However, the pathophysiological mechanism(s) of the expanded (CTG)n repeat remains unclear. Various effects have been proposed, most recently a gain-of-function for mutant DMPK transcripts which results in a generalized RNA metabolism defect, mediated through one or several *trans*-acting proteins involved in RNA processing, and in turn leads to the loss-of-function of multiple genes by qualitatively and/or quantitatively affecting post-transcriptional RNA processing, splicing and/or nuclear export of their transcripts. To test these hypotheses we examined global mRNA expression changes between DM patients and normal controls by comprehensively and simultaneously profiling >6,800 human genes with oligo-based GeneChip microarrays (Affymetrix). Total, nuclear and cytoplasmic RNA fractions of DM patient lymphoblastoid cell lines (four adult-onset, one congenital) as well as primary undifferentiated myoblasts and differentiated myotubes (one adult-onset, one congenital) were profiled. In culture DM myoblasts showed a markedly reduced differentiation rate to myotubes and a tendency to dedifferentiate, suggesting a general block in or reprogramming of differentiation. Expression profiles of DM cell lines differed considerably from controls. Between the different DM cell lines profiled, many of the >6,800 genes assayed showed dysregulation, both down and up. Moreover, comparison of nuclear and cytoplasmic fractions suggested a defect in the nuclear export of some processed transcripts. Interestingly, the number of genes dysregulated and the degree of dysregulation correlated with expansion size. Functions of the dysregulated genes were highly varied. In conclusion, DNA microarray expression profiling identified several novel DM effector candidate genes which may explain the complex pathogenesis of DM.

Program Nr: 1721 from the 1999 ASHG Annual Meeting

The Cornelia de Lange syndrome; Cytogenetic and molecular analysis, with exclusion of linkage to chromosome 3q in some familial cases. *I.D. Krantz[1], E. Tonkin[2], M. Hofreiter[1], M. Smith[2], L. Jukofsky[1], B.P. Conti[1], C. Simpson[2], V. Abraham[1], A.D. Kline[3], T. Strachan[2], L. Jackson[4].* 1) Division of Human Genetics and Molecular Biology, The Children's Hospital of Philadelphia, Philadelphia, PA; 2) Human Genetics Unit, University of Newcastle upon Tyne, UK; 3) Department of Pediatrics, Sinai Hospital of Baltimore, Baltimore, MD; 4) The Division of Medical Genetics, Jefferson Medical College, Philadelphia, PA.

The Cornelia de Lange Syndrome (CdLS) is a complex developmental disorder consisting of upper extremity abnormalities, growth and neurodevelopmental retardation, characteristic facial features, and other anomalies. Most cases of CdLS appear sporadic. Familial cases are rare and demonstrate autosomal dominant inheritance. Partial phenotypic overlap between CdLS patients and patients with duplications of chromosome 3q has been noted. Ireland et al (J. Med. Genet. 28:639-640, 1991) described a patient with classic CdLS and a de novo, apparently balanced, 3;17 translocation with a breakpoint within the dup3q critical region. It has been postulated that a gene within the duplicated region on chromosome 3q is deleted or mutated in patients with CdLS and results in a different but mildly overlapping phenotype. To evaluate the involvement of chromosome 3q in the pathogenesis of CdLS we have studied this region in a large cohort of patients. These studies include; 1) Linkage analysis to the critical region for the dup3q syndrome in 10 familial cases of CdLS, 2) High-resolution chromosome and FISH analysis, with a YAC that crosses the t(3;17) breakpoint, on 30 probands, and 3) Chromosomal analysis of fibroblasts in 9 probands (to evaluate the possibility of a mosaic alteration, not present in lymphocytes). All cytogenetic studies have been within normal limits. Linkage analysis revealed that in 4/10 families haplotypes from 3q were not shared from either parent in the affected individuals, while in 6 families at least one parental haplotype was shared. This implies that this region on 3q26-27 is associated with, at most, a subset of CdLS cases, indicating that this may be a genetically heterogeneous disorder.

Program Nr: 1722 from the 1999 ASHG Annual Meeting

Characterization of a novel autosomal dominant bleeding disorder in a large kindred from East Texas. *S. Kuang[1], M. Phillips[2], Y. Wan[1], P. Thiagrajan[1], D.M. Milewicz[1]*. 1) Int Med Univ of TX Med School Houston, Houston, TX; 2) Centeon Corporation, USA.

A large Texas family with autosomal dominant inheritance of a novel bleeding disorder has been identified. The disorder is characterized clinically by life-threatening bleeding with trauma or surgery and excessive bruising, along with menorrhagia in affected women. Laboratory studies demonstrated prolongation of the PT or aPTT, or both in affected individuals. Paradoxically, assays of known coagulation factors are all within normal limits. To determine the molecular basis of this disease, we undertook a candidate gene linkage analysis in this kindred. Three generations of the family participated in the research. There were 16 affected and 13 unaffected individuals. We initially hypothesized that the cause of the disease in this family could be an antithrombin III mutation that resulted in a constitutively active antithrombin III in the absence of heparin binding. Linkage studies using DNA from the family and an intragenic polymorphic marker within the AT3 gene showed that the disease mapped to this locus on 1q23-25. The coding and intron/exon junctions of the AT3 gene were sequenced using the proband's DNA, but this analysis failed to identify a mutation. Further family members were recruited for the study and 16 polymorphic markers around the AT3 gene were analyzed. Using 2 recombinants, we have narrowed the critical interval for the defective gene to approximately 2 cM. The factor V gene mapped into the disease interval, therefore this gene was sequenced even though factor V activity assays were within the normal range. An alteration was identified in exon 13 of the factor V gene (Ser^{756}), which was present in all affected individuals from this family. This alteration falls within the "B" or hinge region of factor V and is thought to be a private polymorphism in this family. Further studies will determine the genetic defect causing the bleeding disorder in this family and may reveal a novel protein involved in the coagulation cascade.

Program Nr: 1723 from the 1999 ASHG Annual Meeting

Complete mutation screening of the EYA1 gene, genotype-phenotype correlation and genetic heterogeneity associated with Branchio-oto-renal syndrome. *S. Kumar[1], R.J.H. Smith[2], C.W.R.J. Cremers[3], W.J. Kimberling[1]*. 1) Department of Genetics, Boys Town National Research Hospital, Omaha, NE; 2) Department of Otolaryngology, University of Iowa Hospitals & Clinics, Iowa, IA; 3) Department of Otolaryngology, University Hospital Nijmegen, The Netherlands.

Autosomal dominant branchial anomalies associated with hearing loss and renal anomalies affect at least 2% of profoundly deaf children and have estimated prevalence of 1 in 40,000. The clinical features of Branchio-oto-renal syndrome (BOR) consist of external, middle and inner ear malformations, branchial cleft sinuses, cervical fistulas, mixed hearing loss and renal anomalies. The phenotypic expression of BOR can be quite variable even within the same family. The BOR gene has been localized to chromosome 8q13. Recently, a candidate gene EYA1, the human homologue of Drosophila eyes absent gene, was identified and mutations have been reported. In order to evaluate the extent to which mutation in the EYA1 gene contribute to BOR syndrome, we have performed mutation analysis on more than fifty BOR families by heteroduplex followed by sequence analysis of sixteen EYA1 exons. To date, using this approach, we have identified twelve novel mutations. At least 60% of our families, investigated so far, have not shown mutations in the EYA1 gene. Therefore, in the present study, we have also performed genetic linkage analysis on multigenerational BOR type families with 8q markers. Three large BOR type families did not show linkage with 8q markers suggesting the involvement of more than one gene. These families are currently being subjected to genome search. The present results demonstrate genetic heterogeneity. The clinical features of the BOR individuals were also compared to see whether the nature of the mutation correlates with the type and severity of the clinical symptoms. There were no distinct relationships observed between the nature of the mutations and variable clinical features associated with BOR syndrome. These results provide the basis for a molecular-genetic testing that will help the clinical evaluation and genetic counseling of members of BOR families. (Supported by NIH/NIDCD grant #P01 DC01813).

Program Nr: 1724 from the 1999 ASHG Annual Meeting

Identification of 14 new FBN1 mutations in Marfan syndrome and overlapping phenotypes using SSCP and heteroduplex analysis. *M. Lackmy-Port-Lys[1], G. Collod-Beroud[1], G. Jondeau[2], M. Mathieu[3], Y. Maingourd[4], M. Coulon[1], M. Guillotel[1], C. Junien[1,5], C. Boileau[1,5].* 1) INSERM U383, Hopital Necker-Enfants Malades, Paris, France; 2) Service de cardiologie, Hopital Ambroise Pare, Boulogne, France; 3) Centre de Genetique Clinique, CHU d'Amiens, Hopital Nord, Amiens, France; 4) Unite de Cardiologie Pediatrique, CHU d'Amiens, Hopital Nord, Amiens, France; 5) Laboratoire central de Biochimie, d'Hormonologie et de Genetique moleculaire, Hopital Ambroise Pare, Boulogne, France.

Marfan syndrome (MFS) is a dominantly inherited disease of connective tissue. Cardinal manifestations involve the eye, skeleton and cardiovascular systems. The disorder is characterized by considerable variation in the distribution and severity of organ system involvement between families that lead to the definition of diagnostic criteria listed first in the Berlin nosology and subsequently revised in the Ghent nosology. Mutations in the FBN1 gene (15q21.1), encoding fibrillin-1, have been demonstrated to result in MFS and associated phenotypes. We performed molecular analyses of the FBN1 gene in two groups of patients. The first one comprises patients presenting with MFS according to the Berlin criteria. The second group contains patients in which the diagnosis of MFS could not be made with the same criteria but for whom fibrillin implication have been proven by protein analysis, or suspected by segregation of FBN1 markers and exclusion with FBN2 and regional MFS2 markers. Screening of the available gene sequence for further causal mutations has been carried out by searching for single stranded conformational polymorphisms (SSCP) and heteroduplexes on PCR amplified fibrillin DNA isolated from fresh blood lymphocytes. We identified 14 new mutations. Nine are associated with complete forms of MFS [Y1186C, Y1101C, E1158A, 737-1 G->A, T101A, Y1266F, C68R 2, Y458S, G214S], C1081G is associated with neonatal MFS, and 1464-5G->A is associated with an incomplete form of MFS (only ocular and cardiovascular abnormalities). The last one, the G985E mutation, is the first mosaicism identified in a phenotype overlapping MFS (only cardiovascular and skeletal abnormalities).

Program Nr: 1725 from the 1999 ASHG Annual Meeting

Leukodystrophy and oculocutaneous albinism in a child with a 11q14 deletion identified by FISH. *D. Lacombe[1], C. Goizet[1], I. Coupry[1], P. Verin[2], B. Mortemousque[2], L. Taine[1], B. Arveiler[1].* 1) Department of Medical Genetics, Pellegrin University Hospital, Bordeaux Cedex, France; 2) Ophtalmology Department, CHU Bordeaux, France.

The leukodystrophies are a heterogeneous group of rare hereditary diseases affecting the central nervous system (CNS). They dysplay a heredo-degenerative involvement mostly in the white matter and leading to diffuse demyelination of the CNS. In 30% of all leukodystrophies, the causis is unknown and no specific biochemical or genetic abnormality can be identified.

We report a *de novo* chromosomal microdeletion identified by fluorescence in situ hybridization (FISH) in a patient with an unspecified leukodystrophy associated with oculocutaneous albinism (OCA). The patient is the third son of non-consanguineous French parents with unremarkable family history. OCA was noted at birth with total depigmentation of skin, hair and iris associated with nystagmus. Walking and speech were delayed. The visual deficit was evident in early childhood. At the age of 15, generalised epileptic episodes occured with slow activity and paroxysms on EEG. Brain MRI showed white matter lesions typical of leukodystrophy. At age 18, mental retardation was moderate (IQ=51). Metabolic screening was normal. Tyrosinase (TYR) activity was negative in hair roots, leading to classify OCA as tyrosinase-negative. Karyotype on lymphocytes was normal.

A FISH study using a TYR gene probe revealed a heterozygous microdeletion of chromosome 11q14. FISH of both parents was normal confirming the *de novo* aspect of this microdeletion. The 11q14 deletion inactivates one of the two TYR alleles. Sequencing the coding part of the TYR gene revealed a heterozygous mutation of the other allele. This mutation was a previously reported deletion of two bases (G,T) in codon 244/5 producing a stop codon inherited from his father.

These results suggest the localization at chromosome 11q14 of a new type of dominant leukodystrophy. We are now mapping the 11q14 microdeletion using loss of heterozygosity and FISH in order to identify the gene involved in this undetermined leukodystrophy.

Program Nr: 1726 from the 1999 ASHG Annual Meeting

***De novo* mutations in EGF-like repeats of Jagged1 in Alagille syndrome patients.** *P.S. Lai, F.S.H. Cheah, M.H. Liew, M.M. Aw, S.H. Quak.* Dept Pediatrics, National Univ of Singapore, Singapore, Singapore.

Alagille syndrome (AGS) is an autosomal dominant disorder with multisystem involvement. There is variable expressivity of clinical features from apparently normal phenotype to severe cases where liver failure requires transplantation. We have studied five unrelated probands and family members. Three novel mutations were identified within *Jagged1* confirming the involvement of this gene in our patients. All three mutations (1213delC, 2884insA, 2638delCAGT) were present in heterozygous form and occur in the EGF-like repeats of Jagged1. These two frameshifts and one nonsense mutations are predicted to result in truncated protein products without the cystein-rich and transmembrane domains. Mutations in these evolutionary conserved EGF-like motifs of Jagged1 protein is likely to severely affect interaction with the Notch receptor resulting in AGS phenotype in these three patients. Screening of parental samples indicate *de novo* origin of mutations in all three cases. In two of these cases, parents of the probands did not have any clinical features consistent with a diagnosis of AGS while in one, the mother was diagnosed with features of an AGS microform. In the remaining two probands, no truncating mutations could be identified despite screening of all coding exons and flanking introns of *Jagged1*. However, two polymorphisms (1178C®T, 2627A®C) were observed but did not lead to any altered amino acid residues. These sequence alterations were also observed in unaffected family members and show mendellian segregation. It is likely that other unidentified mutations are responsible for the AGS phenotype in these patients.

Program Nr: 1727 from the 1999 ASHG Annual Meeting

Diagnosis of X-linked Myotubular Myopathy by detection of the implicated protein (myotubularin), and genotype/phenotype correlation in mild cases. *j. laporte, f. blondeau, a. buj-bello, c. kretz, j.-l. mandel.* Institut de Genetique et de Biologie Molculaire et Cellulaire (IGBMC), CNRS/INSERM/ULP, 1, rue Laurent Fries, B.P. 163, 67404 ILLKIRCH Cedex, FRANCE.

X-linked Myotubular Myopathy (XLMTM) is a severe congenital muscular disorder characterized by generalized muscle weakness leading in most cases to early postnatal death. The gene (MTM1) responsible for the disease has been isolated and found mutated in most patients (Laporte et al., Nat. Genet. 96). It is about 100 kb in length and composed of 15 exons. It encodes a novel protein named myotubularin that contains the consensus active site of tyrosine phosphatases (PTP). It has been recently reported that myotubularin interacts with several proteins containing the SET domain that are implicated in epigenetic mechanisms of gene regulation (Cui et al., Nat. Genet. 98).

We and others have shown that myotubularin displays a dual specificity phosphatase (DSP) activity. By searching in sequence databases and screening cDNA libraries, we have identified novel members of the MTM1 gene family in different species. We have found 8 MTM-related genes in the human genome and determined the chromosomal localization and expression pattern for most of them. This represents the largest DSP family described so far. A subclass of MTM-related genes lacks the DSP active site and could act as anti-phosphatases. Most missense mutations found in patients affect residues that are conserved in the homologous Drosophila protein. We have obtained antibodies against myotubularin and have used them to detect myotubularin by immunoprecipitation followed by western blot. 90% of the XLMTM patients have no detectable level of myotubularin. This technique could be used for diagnosis and these results confirm that XLMTM is due to a lack of myotubularin activity. Furthermore, we present the identification of novel mutations in the MTM1 gene in patients with severe and mild XLMTM by SSCP and sequencing. Our results implicate mutations in the MTM1 gene in patients with a milder phenotype compared to the typical severe form of XLMTM.

Program Nr: 1728 from the 1999 ASHG Annual Meeting

Gene Therapy for Motor Neuron Diseases by Adenoviral Gene Transfer. *G. Acsadi[1,3], J. Jani[2], R. Anguelov[1], M. Shy[2,3]*. 1) Department of Pediatrics; 2) Department of Neurology; 3) Center for Molecular Medicine and Genetics, Wayne State Univ. Med. School, Detroit, MI 48201.

Spinal muscular atrophy [SMA] and amyotrophic lateral scleroisis [ALS] are caused by a degeneration of motor neurons (MN). Although, the genetic and biochemical background of both diseases has been identified, there is no effective therapy available. Various neurotrophic factors (e.g. GDNF) have the potential to prevent motor neuron degeneration but for an effective treatment, these factors have to be delivered directly to the MN. We have utilized adenovirus vectors (AV) expressing beta-galactosidase (LacZ; as a reporter gene) or GDNF to study the gene transfer into MNs in mice by means of intramuscular or intrathecal delivery. The gene expression was analyzed by histochemical and ELISA techniques. We demonstrate that both LacZ and GDNF genes can be efficiently transferred and expressed in MNs of spinal cord and brainstem by both delivery. After intramuscular administration of AV, the viral genome is transported retrogradely to the MNs as shown by PCR amplification of AV-DNA. The level of gene expression in MN is higher in newborn or immunocompromised mice (FK506 treated, beta2-microglobulin deficient and SCID) compared to control adult mice. Although, at a lower level, the non-immunosuppressed mice are able to maintain significant gene expression (GDNF and LacZ) in the spinal cord or brainstem for at least two month when AV is given intramuscularly. In contrast, after the intrathecally administered AV, the gene expression is lost by one month. In conclusion, AV-mediated gene transfer into skeletal muscles appears a feasible technique to deliver nerve growth factors to MN in spinal cord and brainstem and study the direct effect of these factors in animal models for motor neuron diseases such as SMA or ALS. Supported by Muscular Dystrophy Association of USA.

Program Nr: 1729 from the 1999 ASHG Annual Meeting

Functional analyses of SMN/SMNc gene expression : therapeutic implications in Spinal Muscular Atrophy. *P. Burlet, S. Bertrandy, E. Vial, L. Viollet, A. Munnich, S. Lefebvre.* INSERM U393, IFREM, Hopital Necker Enfant, Paris, France.

Spinal Muscular Atrophy (SMA) is an autosomal recessive disorder characterized by degeneration of motor neurons in the spinal cord and muscular atrophy. SMA is subdivided into three forms according to age of onset and milestones of development. The disease results from alterations of the telomeric copy of Survival Motor Neuron (SMN) gene located in an unstable region of chromosome 5q13 containing a highly homologous copy gene SMNc. SMN is absent in the majority of patients (>95%) and the remaining patients carry intragenic mutations. The SMNc is present in all patients, but absent from about 5%; of control individuals without phenotypic manifestations. A tight correlation between clinical severity of the disease and SMNc protein level was found in tissues and cell cultures derived from SMA fetuses and patients. These findings suggested that the SMNc protein has some active role and pharmacological stimulation of the SMNc gene could represent a therapeutic strategy in SMA. Sequence analyses revealed that SMN and SMNc promoters are identical and that they contain binding sites for several transcription factors. The abundance of binding sites for potential regulatory proteins within the SMN/SMNc minimal promoter suggests that they may be subject to various level of control and regulation in different cellular contexts. Notable among these are responsive elements for cAMP, retinoic acid, mineralocorticoids, hormones, and several cytokines. Since expression of a gene can be greatly influenced by its position in the genome, we investigated directly the SMN protein expression by immunodetection upon stimulation of different cell types. Treatment of lymphoblastoid and fibroblast cell cultures from SMA patients and control individuals with forskolin, INF-b, INF-g, retinoic acid (9-cis and all trans) did not significantly increase the SMN/SMNc protein level. Other stimuli are currently under investigations and will be presented. This approach should identify the key regulators of the SMN/SMNc promoter activity and the pathways responsible for the activation in different cells. Hopefully, these studies may contribute to identify a pharmaceutical agent for SMA.

Program Nr: 1730 from the 1999 ASHG Annual Meeting

Improvement of growth and intelligence in individuals with deletions of 18q treated with growth hormone. *J.D. Cody[1], D.E. Hale[1], M. Semrud-Clikeman[2], P.D. Ghidoni[1], R.L. Schaub[1], N.M. Thompson[4], C.I. Kaye[1], R.J. Leach[1,3]*. 1) Department of Pediatrics, UT Health Science Center, San Antonio, TX; 2) Department of Educational Psychology, University of Texas, Austin, TX; 3) Department of Cellular and Structural Biology, UT Health Science Center, San Antonio, TX; 4) Department of Psychiatry and Behavioral Health Sciences, University of Washington, Seattle, WA.

Most individuals with deletions of 18q have growth failure and mental retardation. We evaluated 60 individuals with 18q deletions and found only 2 with completely normal growth parameters. The remaining individuals had some degree of growth failure. Growth failure due to growth hormone (gh) deficiency severe enough to qualify for gh therapy was found in 66%. We evaluated both gh treated and untreated individuals longitudinally for changes in growth and IQ and have data on the first 14. The period of time between evaluations averaged 30.7 months (range = 14 - 54 months). The height of the participants was compared by determining the number of standard deviations (SD) from the mean for a normal population of the same age. The average height SD of the untreated group (n = 6) was -2.1 and the change in height over the observation period was -0.25 SD. The average height SD for the gh treated group (n = 8) was -3.3 and the change in height SD was +1.8. This demonstrates that the treated children experienced significant catch-up growth while the untreated children maintained a slightly slower than normal growth rate. Over this period of time, the untreated group had IQ changes of between -2 and +6, which are well within the standard error measure for cognitive tests. Performance IQ scores were used since many of these children are hearing impaired and the verbal IQ would underestimate their skills. The treated group had IQ changes of between 0 and +47 points with an average increase of 23 IQ points. An increase of this magnitude is highly significant. The treated individual whose IQ did not increase was the oldest child and had the largest deletion. Future studies will focus on age related responses to treatment as well as on genotypic correlation with response.

Program Nr: 1731 from the 1999 ASHG Annual Meeting

The effects of potential therapeutic agents on ALS-transgenic mice. *N.A Cole, R. Fu, G. Deng, T. Siddique.*
Neurology, Northwestern University Medical School, Chicago, IL 60611.

Transgenic mice over-expressing mutations in SOD1 gene (G1H)develop disease similar to ALS (amyotrophic lateral sclerosis) in humans. Therapeutics can be rapidly screened in this animal model. The thiol lipoate and especially its reduced form dihydrolipoate (DHLA) are potent antioxidants in brain and peripheral nerves. Recently, we administered lipoic acid to our ALS-mice and onset of disease was delayed in females by 13 days. This represents the first observation in ALS transgenic mice of a gender difference which reflects the gender difference in the epidemiology of ALS in humans (1:1.6). Lipoic acid is therefore, another drug that delays the onset of disease in ALS-mice and may provide new insights into the pathogenesis of ALS.

Program Nr: 1732 from the 1999 ASHG Annual Meeting

Application of mutation specific hammerhead ribozymes as gene therapy tools for skin diseases. *P.B. Cserhalmi-Friedman[1], H.C. Dietz[2], A.M. Christiano[1].* 1) Department of Dermatology, Columbia University, New York, NY; 2) Howard Hughes Medical Institute, Johns Hopkins University School of Medicine, Baltimore, MD.

We investigated the feasibility of using target specific hammerhead ribozymes to eliminate selected mRNAs as a possible method of gene therapy in skin conditions. As an experimental model, we used an HPV immortalized human keratinocyte cell line, carrying a 16 bp deletion on one allele of the COL7A1 gene. This mutation leads to the in frame skipping of a 69 bp exon (Cserhalmi-Friedman et al. Lab Invest 1998, 78:1483-1492). We synthesized target specific hammerhead ribozymes to recognize only the mRNA and pre-mRNA transcribed from the mutant allele. The ribozymes were incubated with total RNA extracted from the cells carrying the mutation, and the efficiency and selectivity of the ribozyme action was determined using quantitative RT-PCR to compare the level of the wild-type versus mutant messages. We achieved over 85% selective reduction of the mutant mRNA. To assess the ribozyme activity in cells, we stably transfected the keratinocytes carrying the mutation with the ribozyme expression constructs using a retroviral vector. Then total RNA was extracted, and the ribozyme action was evaluated using the same quantitative RT-PCR system and Northern blot analysis. To prepare for in vivo application, we compared direct liposome mediated delivery, retroviral transduction, and application of naked DNA constructs to express Enhanced Green Fluorescence Protein (EGFP) in mouse skin. For liposome delivery, we used in vitro synthesized EGFP mRNA, for retroviral transduction and for naked DNA uptake we used a construct containing CMV promoter and the EGFP gene. Skin biopsies were taken and analyzed for the expression of EGFP 24-72 hours after the application. Our results demonstrate the feasibility of using hammerhead ribozymes to specifically reduce the level of selected mRNA in the skin.

Program Nr: 1733 from the 1999 ASHG Annual Meeting

Intracellular enhancement of a**-galactosidase A activity in 31 Fabry lymphoblasts and fibroblasts by 1-deoxy-galactonojirimycin.** *J.-Q. Fan[1, 4], S. Nakao[2], C.R. Kaneski[3], R.O. Brady[3], R.J. Desnick[1], Y. Suzuki[4], S. Ishii[5].* 1) Dept Human Genetics, Mount Sinai Sch Medicine, New York, NY, USA; 2) Faculty of Medicine, Kagoshima Univ., Kagoshima, JAPAN; 3) DMNB, NIH, Bethesda, MD, USA; 4) Tokyo Metropolitan Institute of Medical Science, Tokyo, JAPAN; 5) Usuki Bio-Research Center, Oita, JAPAN.

Fabry disease is a genetic disorder of glycosphingolipid metabolism caused by deficiency of lysosomal a-galactosidase A (a-Gal A). This disorder is classified according to clinical manifestations into two groups: 1) classic form with generalized vasculopathy, and 2) cardiac variant of late-onset form with clinical manifestations limited to the heart. Recently, we reported (1) that 1-deoxy-galactonojirimycin (DGJ), a potent competitive inhibitor of a-Gal A, effectively enhanced the enzyme activity in lymphoblasts of a late-onset Fabry patient by cultivation of the cells with DGJ at concentrations lower than normally required to inhibit the intracellular enzyme activity. Oral administration of DGJ to transgenic mice overexpressing a mutant a-Gal A substantially elevated the enzyme activity in major organs. DGJ appeared to accelerate the transport and maturation of the mutant enzyme resulting in increased intracellular enzyme activity. In order to evaluate this strategy with other genotypes and phenotypes, we tested DGJ with 31 strains of Fabry lymphoblasts and fibroblasts that exhibit residual a-Gal A activity. The cells were cultured with 20 mM DGJ at 37°C for 5 days. After washing the cells twice with phosphate-buffered saline, homogenates of the cells were assayed for a-Gal A activity. Among them, 19 cell lines responded to DGJ treatment. Intracellular enzyme activity was elevated from 1.8 - 53 % of normal to 25 - 97 % of normal. The genotypes of these mutations were E59K, E66Q, I91T, A97V, R112H, F113L, N215S, Q279E, M296I, M296V, R301Q and R356W. Most of the patients were diagnosed as the cardiac late-onset Fabry disease. Administration of DGJ may be considered as a therapeutic possibility for patients with the late-onset form of Fabry disease. (1) Fan, J.-Q., Ishii, S., Asano, N. and Suzuki, Y. *Nature Med.* **5**, 112-115 (1999).

Program Nr: 1734 from the 1999 ASHG Annual Meeting

Inhibitors of 4-OH phenylpyruvate dioxygenase and adenoviral gene therapy for the treatment of murine alkaptonuria. *J.M. Fernandez-Canon, K. Manning, M. Al-Dhalimy, M. Grompe.* Molecular and Medical Genetics, Oregon Health Sci. University, Portland, OR.

Alkaptonuria is a inborn error of metabolism caused by deficiency of homogentisic acid dioxygenase (HGD) an enzyme in tyrosine catabolism. The disease phenotype has a late onset and is characterized by degenerative arthritis caused by accumulation of homogentisic acid (HGA) metabolites (ochronosis). To date therapeutic interventions such as tyrosine restriction have not been successful in preventing disease progression. Recently, a new class of compounds consisting of electrophilic benzoyl derivatives of 1,3 cyclohexane dione has been identified. These drugs are potent inhibitors of 4-OH phenylpyruvate dioxygenase, the enzyme upstream of HGD in tyrosine metabolism. We have tested 2 such compounds, 2-(2-nitro-4-trifluoromethylbenzoyl)-cyclohexane dione (NTBC) and 2-(2-chloro-4-methanesulfonylbenzoyl)-cyclohexane dione (CMBC), as therapeutic drugs in a murine model of alkaptonuria. HGD deficient mice were given different doses of NTBC or CMBC in their drinking water and their urine HGA and blood tyrosine levels were measured. While excretion of urinary HGA could be completely eliminated by this approach, blood tyrosine levels became elevated. Therefore treatment of alkaptonuria with blockers of 4-OH phenylpyruvate dioxygenase represents a trade-off between high blood tyrosine levels and the accumulation of arthritis-causing HGA metabolites.

For this reason liver directed gene transfer of HGD may be a more attractive approach to the treatment of HGD. We have generated a first generation adenoviral vector expressing the human HGD and are currently testing this vector in vivo in our murine model. The 2 approaches to the treatment of alkaptonuria will be compared.

Program Nr: 1735 from the 1999 ASHG Annual Meeting

Adeno-associated virus mediated gene therapy for the neurological disorder of mucopolysaccharidosis III B using a knock-out mouse model. *H. Fu[1], T.J. McCown[2,3], R.J. Samulski[3], J. Muenzer[1].* 1) Department of Pediatrics, University of North Carolina, Chapel Hill, NC; 2) Department of Psychiatry, University of North Carolina, Chapel Hill, NC; 3) Gene Therapy Center, University of North Carolina, Chapel Hill, NC.

Mucopolysaccharidosis type III B (Sanfilippo syndrome type B, MPS III B) is due to the deficiency of the lysosomal enzyme a-N-acetylglucosaminidase (NaGlu). MPS III B is characterized by severe central nervous system (CNS) involvement, but with only mild somatic disease. Correction of lysosomal storage in CNS is critical for the successful treatment of the MPS III B patient. A MPS III B knock-out mouse model (Li et al, AJHG 63: A15, 1998, obtained from Dr. EF Neufeld, UCLA) was used in this study to investigate the feasibility of adeno-associated virus (AAV) mediated gene therapy for treating the CNS diseases in MPS III B patients. Two therapeutic AAV vectors were constructed, containing the human NaGlu cDNA, driven by a human cytomegalovirus (CMV) promoter or the neuron specific enolase (NSE) promoter. In vitro experiments showed that AAV-CMV-NaGlu could mediate NaGlu expression in both Hela and MPS III B fibroblast cell lines, where the recombinant NaGlu was secretory. The AAV vectors were delivered into 4 brain structures of a MPS III B knock-out mouse model by direct microinjection (10^6 viral particles/site). Efficient expression of functional NaGlu was detected in inferior colliculus, hippocampus, hypothalamus and cortex for up to 12 weeks. Our data demonstrates that AAV-mediated gene delivery can result in enzyme expression in different brain structures of MPS III B mice, which may be sufficient for correction of lysosomal storage in these areas. (This project was supported by a grant from The Children's Medical Research Foundation, Inc., Western Springs, IL).

Program Nr: 1736 from the 1999 ASHG Annual Meeting

Cessation of Enzyme Replacement Therapy (ERT) in Gaucher Disease Type 1. *E.D. Geller, K.A. Grinzaid, L.J. Elsas.* Division of Medical Genetics, Dept of Pediatrics, Emory Univ, Atlanta, GA.

Intravenous replacement of mannans-engineered b-glucosidase is an expensive but effective treatment for Gaucher disease. There are few reports regarding the effect of discontinuing ERT. Because ERT is expensive and time consuming, four of our patients who experienced clinical improvement during their initial treatment with ERT chose to discontinue ERT under careful observation for 1 or more years.

Genotype	N370S/L444P	N370S/L444P	K79N/K79N	N370S/N370S
Age@dx/trt(yrs)	5/6	5/13	19/49	35/51
On/Off trt-yrs	On-3/Off-1/On-1	On-2/Off-1.5/On-1	On-2/Off-2/On-1	On-1.5/Off-3
Liver/%D/yr	-16/ +25/ -8	-8/ +3/ -16	-22/ +7/ -16	-10/ +2
Platelets/%D/yr	+26/ -23/ -7	+4/ -8 / +10	+35/ -21/ +55	+82/ -12
MRI index/%D/yr	not available	+25/ -5/ n/a	+101/ -15/ n/a	+10/ +9

All patients improved on ERT, and when ERT was interrupted all had progressive decline in clinical status. The patient with the genotype N370S/N370S declined very slowly over the 3 year period. His spine bone density was decreasing on and off ERT , but is responding to Fosamax off ERT. The other three patients were restarted on ERT within 2 years of cessation. Resumption of ERT resulted in improvement of visceral and hematological parameters within one year. We conclude that cessation of ERT results in slow decline of visceral and hematological parameters, but that reinstitution of ERT corrects these losses. There is a positive correlation between severity of disease(based on genotype and age of diagnosis)and response to cessation of ERT.

Program Nr: 1737 from the 1999 ASHG Annual Meeting

Cooperability of SP1 and GABP transcription factors in activating the Utrophin promoter : Implications for functional substitution therapy of Duchenne muscular dystrophy (DMD). *M. Gyrd-Hansen[1], T. Krag[1], J. Shang[2], A. Rosmarin[2], T. Khurana[1]*. 1) Clin. Biochemistry, Glostrup Hospital, Denmark; 2) Div. Hematology, Miriam Hospital, Providence, USA.

Utrophin functional substitution is a strategy designed to circumvent problems associated with gene therapy in DMD and relies on using trans-acting factors to activate the utrophin promoter and the ability of high levels of utrophin to functionally substitute and rescue dystrophin-deficient muscle. Recently we had demonstrated that the utrophin promoter could be activated by the GABP transcription factor complex via heregulin mediated ERK activation. However, the magnitude of increase in utrophin expression using these methods (2.4X) is modest. Here we demonstrate that the transcription factor SP1 activates the utrophin promoter. Activation is an order of magnitude greater than previously observed. We have defined two regions of the promoter that are putative SP1 sites based on structural predictions, gel and super-shift assays as well as DNAse hypersensitivity. Using site-directed mutagenesis and reporter assays we have analyzed the relative contribution of the proximal and distal sites. Co-transfection using both GABP and SP1 reveal that these transcription factors cooperate and activate the utrophin promoter to a greater extent than ascribable to a simple summation of each factor's individual contribution. Our results suggest that cooperability of GABP and SP1 are major determinants in transcription factor mediated recognition, recruitment and remodeling of the utrophin gene promoter that modulates utrophin expression in muscle and methods that stabelize this interaction in vivo, may be utelized to achieve high levels of utrophin expression in DMD patients.

Program Nr: 1738 from the 1999 ASHG Annual Meeting

Cell Lines for Production of Gutted Adenovirus. *D.J. Hartigan-O'Connor[1,3], C. Barjot[2,3], J.S. Chamberlain[1,2,3].* 1) Program in Cellular and Molecular Biology; 2) Department of Human Genetics; 3) Center for Gene Therapy, University of Michigan, Ann Arbor, MI.

Gutted, or helper-dependent, adenoviral vectors offer some advantages over conventional Ad vectors. Because gutted viruses contain no viral genes, their cloning capacity is much larger than that of conventional vectors and the possibility of a harmful host immune response is reduced. Unfortunately, these vectors have proven difficult to grow and purify. We report progress in our efforts to develop cell lines that facilitate production of gutted Ad vectors. C7 cells are 293 cells that stably express both adenovirus DNA polymerase and preterminal protein. We have previously shown that C7 cells plaque suboptimal Ad genomes with increased efficiency, which leads to increased yield of gutted virus. We now show that modified versions of the C7 line plaque adenovirus with still higher efficiency and we suggest applications for such cell lines. We find that C7 cells expressing Cre recombinase, when used in combination with helper viruses containing "floxed" packaging signals, provide a significant increase in gutted viral yield and purity at each serial passage. At early growth stages, a single passage on C7 Cre cells provided an average 20-fold increase in both gutted viral yield and purity. We created a helper virus that contains a mutation in the Ad polymerase gene in addition to a "floxed" packaging signal. Use of this virus increases the purity of gutted viral preparations but reduces yield somewhat when compared with pol-positive helpers. This floxed, E2B-deleted helper should reduce the danger of an immune response directed against residual helper virus. Comparison of a variety of Cre lines reveals significant variation in Cre levels and excision efficiency, a factor that may be important for rapid production of gutted virus.

Program Nr: 1739 from the 1999 ASHG Annual Meeting

Gene therapy for twitcher mouse using retrovirus vector. *K. Inui, C. Kokubu, T. Nishigaki, T. Muramatsu, H. Tsukamoto, N. Sakai, S. Okada.* Develpomental Medicine (Pediatrics), Graduate School of Medical Science, Osaka Univ, Osaka, Japan.

Krabbe disease (globoid cell leukodystrophy) is an autosomal recessive neurodegenerative disorder that affects both the central and peripheral nervous systems due to an enzymatic defect of galactocerebrosidase (GALC). Twitcher mouse (TW), naturally occurring mouse model for Krabbe disease, provides an excellent experimental model for gene therapy. In this study to examine the effectiveness of gene therapy using retrovirus vector targeted to bone marrow (BM) cells, recombinant retrovirus vector containing human GALC cDNA was constructed. The normalization of GALC metabolism was confirmed in transfected cultured skin fibroblasts from Krabbe disease and TW. Then, TW bone marrow cells were transfected ex vivo and they were transplanted intraperitoneally into twitcher neonates at 2 postnatal days. This treatment significantly increased body weight at 26 days and 35 days compared to untreated, busulfan treated and busulfan+BM transplantation from TW groups. Human GALC cDNA was not detected in bone marrow cells but sciatic nerve, and the GALC activities were significantly increased in sciatic nerves around 40 postnatal days. However, survival length did not change. The transduction efficiency was about 20 % in hematopoietic colonies. The low efficiency of transduction and pretreatment of BM using busulfan were not enough to extend the survival, but differentiated macrophages from stem cells infiltrated into demyelinating sciatic nerve and stayed there as tissue macrophages. This is the first report of gene therapy for TW using retrovirus vector. There is a possibility to improve the survival of TW by increasing transduction efficiency and using high expression vectors.

Program Nr: 1740 from the 1999 ASHG Annual Meeting

Clinical accessment of therapeutic response to enzyme replacement therapy in 16 Korean Gaucher pts. *H.J. Kim[1], M.J. Ha[1], J.H. Cho[1], B.S. Kim[1], M.K. Kim[1], S.H. Kim[1], H.S. Kim[1], J.S. Kim[1], Y.A. Lim[1], Y.J. Lee[1], H.W. Yoo[2], C.H. Park[1]*. 1) Ajou Univ. School of Medicine, Suwon, Korea; 2) Ulsan Univ. College of Medicine, Seoul, Korea.

16 Korean Gaucher dis. pt.(9 type 1 and 7 type 3), aged 1 to 25 yrs old, began on Enzyme Replacement Therapy (ERT) using Cerezyme with starting dosage of 30u/kg/2wks since May 1998, except 3pts. of type 1, previously on Ceredase from 1 to 3yrs, switched to CZ. Clinical accessment is made on general growth parameters, hematologic parameters of Hb. platelet count, liver function test and acid phosphatase, viceral involvements of hepatosplenomegaly by MRI, skeletal involvements by plain X-ray and MRI and neurologic evaluation with EEG prior to and every 6 m. after ERT (for 1 to 5 yrs follow up). 2 pts. developed antibody against CZ; 1 y.o. pt. developed generalized urticaria after 3 m. Rx. with CZ, massive hepatosplenomegaly and pancytopenia, requiring splenectomy and switched to CD. The other 2 y.o. tolerated the CZ Rx. without clinically adverse reaction. All but 3pts. have responded well and maintained on initial dosage of 30u/kg/2wks regimen; 2 type 3B, required 30u/kg/wk regimen and 1 type 1, to 60u/kg/2wks after recurrent femur Fx. In addition to most freq. finding of subjective "feeling of well being, ", the most consistant finding was the linear growth, evident in all prepubertal children, esp 6 with short stature (Ht.<3%), with growth spurt following 6 m. of Rx., althrough the radiologic bone improvement was not evident yet. Changes of Hb and and platelet count are not informative in 10 splenectomized pts. but acid phosphatase level fell down 20% in 6 m., and 30-50% in 1yr. The reduction of spleen and liver size was most evident in 6 month (-30%) but subsequent reduction was not big as the 1st yr. 5 type 3A pts. responded well on ERT with no further progression in CNS S//S except a 16 y.o. female who had progressive myoclonic seizures since 13 y.o. requiring BMT. With high dosage and high frequency regimen (30u/kg/wk), two type 3B pts. responded well for failure to thrive, massive splenohepatomegaly and other hematologic abnormalities, but abnormal ocular movements have not changed yet.

Program Nr: 1741 from the 1999 ASHG Annual Meeting

Recombinant AAV vectors integrate as single copies even in the presence of wt-AAV. *M.J. Landrum, G.R. Cutting.* Dept Human Genetics, Johns Hopkins Univ, Baltimore, MD.

Wild-type AAV (wt-AAV), a potential gene therapy vector, has the unique property of integration into a specific region of human chromosome 19 termed AAVS1. The integrated provirus is usually found in multiple tandem copies in head-to-tail fashion. Although the precise mechanism of site-specific integration is unknown, the wt-AAV Rep protein plays a major role. Rep^- recombinant AAV (rAAV) vectors integrate to non-19 sites and adding back Rep protein as a plasmid can restore targeting. To study Rep- and non-Rep-mediated integration, we infected cells with both wt-AAV and rAAV or with rAAV alone to test the hypothesis that the presence of Rep protein provided by wt-AAV allows rep^- rAAV to integrate in a manner similar to wt-AAV. Cells were infected either with AAV-GFP, which contains the green fluorescent protein (GFP) and neo-resistance (neo) genes, or with both wt-AAV and AAV-GFP. Colonies were selected for GFP expression and G418 resistance. One single infection clone and two from the dual infection were chosen for extensive Southern blot analysis of the AAV-GFP integrations. Digestion with Hind III which does not cut AAV-GFP and hybridizing with an AAVS1 probe showed that none of the clones has an integration disrupting AAVS1. Five restriction enzymes and three AAV-GFP probes confirmed that an intact viral genome is present. Novel hybridizing bands representing the flanking sequences were used to construct a restriction map of the flanking DNA. The maps confirmed that each clone contains a single copy of integrated AAV-GFP, regardless of the presence of wt-AAV. Fourteen more dual infected clones were characterized. Hind III digestion and hybridizing with AAVS1 confirmed that none of the AAV-GFP integrations disrupt AAVS1. Digestion with an enzyme that yields neo and 3' flanking sequence on a single fragment shows that 13 of 14 clones have a single copy of AAV-GFP integrated at a unique site. These results indicate that in contrast to wt-AAV, rAAV vectors do not specifically target chromosome 19 and integrate as single copies, suggesting that rAAV uses a different non-Rep mediated mechanism of integration, even in the presence of wt-AAV.

Program Nr: 1742 from the 1999 ASHG Annual Meeting

Correction of the metabolic defect in Fabry mice through genetic modification of depot organs. *C. Li[1], R. Ziegler[1], M. Cherry[1], P. Berthelette[1], H. Romanczuk[1], R.J. Desnick[2], Y.A. Ioannou[2], N.S. Yew[1], S.H. Cheng[1]*. 1) Genzyme, Framingham, MA; 2) Mt. Sinai Sch. Med., NYC, NY.

Fabry disease is caused by a deficiency of the lysosomal enzyme a-galactosidase A (a-Gal) resulting in deposition of globotriaosylceramide (GL-3) in vascular lysosomes. Normally, a portion of the enzyme is secreted into circulation which can be recaptured by distant cells through specific receptors. Therefore genetic modification of a depot organ such as liver or lung may suffice to produce corrective levels of the deficient enzyme. Previously, we demonstrated the efficacy of systemic administration of an adenovirus vector encoding a-Gal in Fabry mice. Injection of 10^{11} particles of Ad2/CMVHIagal resulted in high levels of a-Gal in all organs examined, reducing GL-3 to near normal levels. However, associated with administration of this dose of recombinant virus was a robust host immune response which obviated readministration and attenuated expression. We showed transient immunosuppression using the antibody MR1 or deoxyspergualin could overcome this block to readministration and facilitated greater persistence of expression. An alternative to intravenous administration is delivery to the lung. This mode offers the advantage of localized delivery and perhaps greater ease of immunosuppression. Pulmonary instillation of 10^{10} particles of Ad2CMVHIagal into BALB/c mice resulted in expression of a-Gal in the lung, liver, spleen, kidney and plasma. The observation of enzyme outside of the lung suggests that it could cross the blood-air barrier, enter systemic circulation and be internalized by distal tissues. However, expression was transient and undetectable after 3 weeks. The loss of expression was likely due in part to an immune response to the adenovirus and transgene product. Fabry mice have been treated to determine whether this low level of enzyme is sufficient to reduce accumulated GL-3. In addition, an AAV vector encoding the hydrolase has been constructed as this vector purportedly facilitates greater longevity of expression. Comparative studies of these two vector systems will aid in our selection of the most appropriate vehicle for use in gene therapy of Fabry disease.

Program Nr: 1743 from the 1999 ASHG Annual Meeting

Phenotypic correction of feline lipoprotein lipase (LPL) deficiency by adenoviral gene transfer. *G. Liu[1,3], K.J.D. Ashbourne Excoffon[1,3], J.E. Wilson[2,3], B.M. McManus[2,3], L. Miao[1,3], M.E.S. Lewis[1,3], M.R. Hayden[1,3].* 1) Dept. of Medical Genetics; 2) Dept. of Pathology; 3) University of British Columbia, Vancouver, B.C. Canada, V6T 1Z4.

Previous studies have revealed that adenovirus-mediated ectopic liver expression of human LPL (huLPL) can efficiently mediate plasma triacylglycerol (TG) catabolism in mice despite its native expression in adipose and muscle tissue. We aimed to explore the feasibility of liver-directed gene transfer and enzyme replacement for human LPL deficiency using a larger, naturally occurring feline animal model of complete LPL deficiency, remarkably similar in phenotype to the human disorder. A cohort of LPL-deficient (LPL -/-) cats were given iv 8x109 pfu of a CMV promoter/enhancer-driven, E1/E3-deleted adenoviral vector containing a 1.36 kb huLPL cDNA (Ad-LPL) or reporter alkaline phosphatase gene (Ad-AP). Active circulatory huLPL was readily released after iv heparin injection resulting in a ten-fold reduction in plasma TGs, disappearance upon FPLC of the plasma VLDL/chylomicron carriers of TG and enhanced clearance of an excess iv fat load. Antibody against huLPL protein was detected at Day 14 with adenovirus-specific neutralizing antibody present 7 days post gene transfer in both cat cohorts. Tissue-specific expression of the huLPL transgene relative to controls was confirmed by RT-PCR. While huLPL expression was greatest in the liver, other tissues including spleen and lung expressed huLPL message, in direct correlation with histopathological evidence of increased Oil red O positive neutral lipid influx. In contrast, intravenous LPL enzyme replacement therapy (ERT) lead to rapid disappearance of 900 mU/kg of active bovine LPL enzyme from the circulation, with T1/2 occurring at < 10 min in 2 LPL -/- cats. Heparin injection one hour later released < 10% of the original bovine LPL, further indicating its rapid systemic clearance, inactivation or degradation as well as its ineffectiveness as a viable therapeutic alternative for complete LPL deficiency.

Program Nr: 1744 from the 1999 ASHG Annual Meeting

In vivo gene therapy of Pompe disease using an adenoviral vector. *E. Martin-Touaux[1], K. Azibi[1], J.P. Puech[1], D. Chateau[2], E. Kremer[3], N. Raben[4], M. Fardeau[2], A. Kahn[1], L. Poenaru[1].* 1) Laboratoire de Genetique, INSERM U129, CHU Cochin, Paris, France; 2) U153, Pitie-Salpetriere, Paris, France; 3) Genethon, Evry, France; 4) NIH, Bethesda, USA.

Pompe disease (glycogen storage disease type II or GSD II) is an autosomal recessive disorder caused by a deficiency of the lysosomal acid a-glucosidase (GAA). Patients with GSD II accumulate glycogen in lysosomes resulting in three clinical phenotypes: infantile, with severe cardiomyopathy leading to death before 2 years of life, juvenile and adult with a progressive skeletal muscle weakness affecting also the diaphragm causing respiratory failure. No treatment was available for this disease so far. Recently, different therapeutic approaches have been considered such as enzyme replacement and gene therapy. M. Nicolino *et al* in our laboratory have obtained a first generation recombinant adenoviral vector containing the GAA cDNA under the control of the cytomegalovirus promoter (AdGAA). This vector was able to transduce fibroblasts, myoblasts and myotubes of GSD II patients, to provide a high level expression of the GAA and to correct glycogen accumulation. In the present study, we aim to demonstrate the efficiency of the GAA adenoviral vector in vivo in a mouse model of the disease. Previously, we demonstrate the ability of a control adenoviral vector encoding the b-galactosidase, to transduce efficiently the injected muscle by intramuscular injection, and the liver but also the heart and a few skeletal muscle fibers after intravenous injection. The expression is still present 2 months later. The GAA adenoviral vector was administered in newborn mice in the same targeted tissues. Acid a-glucosidase activity was measured 15 days or 2 months after injection and the clearance of intracellular glycogen was evaluated by direct assay and histological analysis, especially electron microscopy. The AdGAA vector permits to restore the GAA activity in liver and muscle. The activity seems to be sufficient to hydrolyse the intralysosomal glycogen. These results are the first step in the evaluation of the feasibility of gene therapy in Pompe disease using a specific recombinant adenoviral vector.

Program Nr: 1745 from the 1999 ASHG Annual Meeting

A family with autosomal dominant polyposis coli and Cowden disease unlinked to the APC, PTEN, and HMPS loci : A new cancer syndrome. *H. Mehenni[1], R. Chergui[2], M.A Brundler[3], S.E Antonarakis[1], M.A Morris[1]*. 1) Medical Genetics, Centre Med Univ, Geneva, Switzerland; 2) Service de gastroenterologie, clinique D. Rahmouni, CHU Algiers, Algeria; 3) Division of Pathology, department of Internal medecine, Cantonal University Hospital of Geneva, Geneva, Switzerland.

Various inherited polyposis syndromes predispose to the development of colonic polyps and colorectal cancer; the elucidation of the molecular pathogenesis of these syndromes is furthermore of great value in understanding mechanisms of sporadic tumorigenesis. We studied a four-generation family of 28 individuals, eight of whom were affected by adenomatous polyposis coli. At colonoscopy, there were more than 100 polyps, which were adenomatous. There were no extra-colonic symptoms except CHRPE in one case. Five individuals developed cancer (three colorectal and one small intestinal and one nasopharynx cancer) and among them four died. A further family member presented with hamartomatous polyps plus cutaneous hamartomas and a thyroid tumor (Cowden disease). Seven family members were unaffected, and twelve of unknown status. The transmission of the polyposis was apparently autosomal dominant. Genetic linkage analysis using microsatellites tightly linked to three genes mutated in polyposis coli, APC, PTEN, and HMPS, showed significant negative Lod scores compatible with exclusion of linkage of the phenotype in this family to these loci. The clinical and genetic data in this family suggest that this syndrome (Cowden/FAP association) has a new genetic etiology. We term this family's disease Hereditary Associated Polyposis Syndrome (HAPS). The main characteristic of this syndrome is the high occurrence of cancer. A genome-wide search in this family will provide the mapping position of the locus and help in the cloning of the gene.

Program Nr: 1746 from the 1999 ASHG Annual Meeting

Incidence and functional consequences of microsatellite instability in a population series of young endometrial cancer patients. *A.L. Millar[1], C. Luceri[1], E. Holowaty[2], S. Gallinger[1], M. Redston[1], B. Bapat[1]*. 1) Center for Cancer Genetics, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, University of Toronto, Toronto, ON, Canada; 2) Cancer Care Ontario, Toronto, ON, Canada.

Endometrial adenocarcinoma (EC) is the most common noncolorectal cancer in women with familial cancer syndrome, HNPCC. The frequency of HNPCC in the population varies widely, possibly due to incomplete penetrance of the disease, variable phenotype and empirically established clinical criteria (the Amsterdam criteria); which do not account for extra-colonic cancers including EC. Cancers arising in HNPCC patients display microsatellite instability (MSI), caused by genetic defects in mismatch repair (MMR) genes. Germline defects in two MMR genes, MSH2 and MLH1, account for 70% of known HNPCC families. Based on the extent of instability in colon cancers, three classes of MSI are recognized; MSI-H (high, >40% instability), MSI-L (low, <40%) and MSS (stable). We have undertaken a molecular genetic and epidemiologic study of the population incidence of HNPCC-associated EC, using the Ontario Cancer Registry (OCR). All incident cases of young (<50 yrs) EC within the geographical region of Central-East Ontario during 1989-1993 are identified using OCR (n=185). Hematoxyline & Eosin stained sections (5 micron) from all EC cases are reviewed and areas of >75% neoplastic cellularity are selected for microdissection. Matched normal and tumor DNA is screened for MSI using a panel of 5 microsatellite loci (D2S123, D5S346, D17S250, BAT25, BAT 26) and MSI positive cases subjected to immunohistochemical analysis of MSH2 and MLH1 expression, in order to identify underlying mismatch repair gene defect. Of 118 cases analyzed to date, 36 (30.5%) were MSI-H, 13 (11%) were MSI-L and 69 (59%) were MSS. BAT 26 was identified to be the most useful marker for MSI analysis of EC and exhibited 88% sensitivity and 95% specificity.

Program Nr: 1747 from the 1999 ASHG Annual Meeting

A model database for the cataloging of APC mutations and clinical features: Providing a global forum for accurate genetic information. *G.A. Miller, A. Whetsell, M. Wright, R.J. Pomponio.* Molecular Profiling Laboratory, Genzyme, Framingham, MA.

The HUGO Database Initiative has made recommendations for standardized mutation nomenclature, formation of locus-specific and ethnic/national mutation databases, and advocated the creation of peer-reviewed "electronic journals" for mutation reports. Additionally they have been strong proponents of bioinformatics software development and have offered a forum for the discussion and showcasing of new curatorial programs. Recently the paper "Guidelines and recommendations for content,structure, and deployment of mutation databases" (Scriver et al. 1999 Hum Mutat 13:344-350) was published as a compilation of these discussions. In addition to the research utility of such databases it is clear that future genetic testing programs will require accurate, user friendly and up-to-date sources of clinical genetic information.

Consistent with these recommendations, we have developed a model database for the accessioning and reporting of mutations within the Adenomatous Polyposis Coli gene (APC) using the commercially available FileMaker Pro software package (FileMaker, Inc. Santa Clara, CA). The database allows for a more complete "event-based" record of both the molecular and phenotypic data associated with each mutation either reported in the literature or through web-based submission to the site. Unique allelic variants are assigned a permanent accessioning number allowing for efficient searches and sorting of related data. The database can seamlessly integrate data from disparate sources and can be linked to a number of currently existing generalized information databases such as OMIM, GenBank and GDB.

We are currently inviting a dialogue between individuals and institutions working with APC towards creating an APC Consortium for the open reporting and discussion of mutations in this gene. The ultimate goal of this effort is to provide an open, robust, accurate resource for the research and genetic testing communities.

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Program Nr: 1748 from the 1999 ASHG Annual Meeting

Nipple Aspiration Fluid(NAF): A "Pap Smear" for the Breast in *BRCA* 1/2 Carriers? *G. Mitchell[1,2], P.A. Trott[2], N. Coleman[3], L. Morris[3], E. Sauter[4], R.A. Eeles[1,2].* 1) Institute of Cancer Research, Sutton, Surrey, UK; 2) Royal Marsden Hospital, London, SW2,UK; 3) Addenbrookes Hospital,Cambridge, CB2 2QQ,UK; 4) Thomas Jefferson University, Philadelphia 19107.

Mammographic breast cancer screening is advised for *BRCA*1/2 mutation carriers, but concerns remain as to reduced premenopausal mammographic sensitivity and radiation exposure. An alternative technique is desirable. NAF is produced by ductal epithelial cells, cells which undergo malignant transformation, and contains exfoliated epithelial cells and other cell types. NAF analysis is not new, but an historically poor aspiration rate of ≤60% rendered this technique an unsuitable screening method. **Purpose** To assess the aspiration success rate and normal NAF cellular profile from serial aspiration in non-carriers prior to the start of a NAF screening study for *BRCA*1/2 mutation carriers. **Methods** 15 healthy premenopausal female volunteers had NAF taken from both breasts weekly for 2 menstrual cycles. NAF preparations were stained with H&E and Pap stains, epithelial membrane antigen (EMA) and CD68 (macrophage marker). Cellular proliferative activity was assessed in cycle 2 using an antibody against minichromosome maintenance protein-2 (Mcm-2), a member of the pre-replication complex. **Results** A successful aspiration rate of 88%(247/280 breasts). Of 189/247 specimens available for cytology 92% were cellular, 47% contained epithelial cells and all cellular specimens contained foam cells. Foam cells expressed EMA and CD68 suggesting a heterogeneous population with epithelial and macrophage differentiation. Cellular content did not show menstrual variation or reduce on serial sampling. In 24/104(23%) of samples foam cells expressed Mcm-2 and in 3/24 Mcm2 was also expressed in epithelial cells. Positive cases were from 6 women. No consistent menstrual cycle correlation of Mcm-2 was detected. **Conclusions** NAF cytology maybe useful as a breast cancer screening method similar to the Pap smear, particularly if a subset of foam cells demonstrate epithelial origin. The sensitivity of this method maybe increased by the use of proliferation markers as suggested for cervical Pap smears. Funding by Breast Cancer Campaign.

Program Nr: 1749 from the 1999 ASHG Annual Meeting

Androgen receptor gene mutation R726L is associated with prostate cancer in Finland. *N. Mononen[1], P.A. Koivisto[1], J. Schleutker[1], M. Matikainen[1], T. Tammela[1], J. Trapman[2], O-P. Kallioniemi[3].* 1) Lab. Cancer Genetics, Inst. of Medical Technology, Tampere, Finland; 2) Dept. of Pathology, Erasmus University, Rotterdam, the Netherlands; 3) Cancer Genetics Branch, NHGRI, NIH, USA.

Due to the importance of androgen signaling in prostate cancer, the role of androgen receptor has been extensively studied as a risk factor for prostate cancer. However, only few germ-line androgen receptor mutations have so far been found, none of which have been conclusively linked with the disease. We found a recurrent germline mutation at codon 726 of the androgen receptor gene (AR) in Finnish prostate cancer patients. This missense mutation (G>T) replaces arginine with leucine in the hormone-binding domain of the receptor, and has previously been shown to alter transactivational properties of the receptor (Elo et al, J Clin Endocrinol Metab. 1995 Dec;80(12):3494-500). To explore the disease association of R726L, we performed ASO-analysis of 417 consecutive prostate cancer patients and 562 healthy male and female blood donors (altogether 684 AR alleles) collected at the Tampere University Hospital, as well as 107 Finnish prostate cancer families. R726L was significantly more common among unselected prostate cancer patients (8/417, 1.9%; p=0.025, RR=4.4) than the carrier frequency in blood donors (3/684, 0.4%). Interestingly, R726L was also present in three prostate cancer families (2.8%; p=0.038, RR=6.2). In two of these families, segregation of R726L with disease could be demonstrated. In conclusion, R726L is associated with prostate cancer and present in up to 2%; of Finnish prostate cancer patients, as well as a subset of prostate cancer families. R726L and other similar functional AR SNPs require further attention as predisposing or modifier loci for human prostate cancer.

Program Nr: 1750 from the 1999 ASHG Annual Meeting

A New Insertional Mutation and Differentially Spliced mRNAs in the BRCA1 Gene. *M. Munnes, I. Zuther, B. Schmitz, W. Doerfler.* Inst Genetics, Univ Cologne, Koeln, Germany.

In breast cancer, one of the most frequent cancer forms in women, one major risk factor besides progressing age and late pregnancy is a family history of breast and/or ovarian cancer. Among familial breast cancer patients, early onset (<30 years), bilateral disease as well as joint occurrence with ovarian cancer are more frequent compared to the general population. Two major candidate genes, the BRCA1 (17q21) and BRCA2 (13q12.3) have been identified. Numerous mutations in the 24 exons of the BRCA1 gene that spans a genomic region of 100 kbp have been found in patients. We have identified a family with several cases of breast/ovarian or other forms of cancer in three generations and an index patient with breast cancer diagnosed at age 26. DNA and mRNA from peripheral white blood cells have been isolated and all PCR amplified exons have been subjected to automated DNA sequencing. Within the coding sequence we found two polymorphic nucleotide exchanges in exons 11 and 16 which seemed unrelated to the disease. More importantly, we detected a so far undescribed 10 bp heterozygous insertion in exon 6 of the BRCA1 gene. This insertion alters and truncates the BRCA1 protein after 86 amino acids. The cDNA sequences obtained by RT-PCR displayed biallelic expression of the BRCA1 gene. By protein truncation tests (PTT), we also confirmed the truncation of the BRCA1 protein due to the insertion of a premature stop codon in the DNA sequence in this woman. Haplotype analyses and DNA sequencing revealed the transmission of the 10 bp insertion from the patient's mother who had also been treated for breast cancer. In studies on the transcription patterns of the BRCA1 gene, we have noted several splice variants in the 5'-part of the gene affecting exons 3, 5 and 8. In about 50 % of all transcripts the first codon of exon 8, coding for glutamine, was absent. The complete or partial loss of exon 3 or 5 led to the disruption of the RING finger motif in the c-terminal part of the BRCA1 protein. PTT analyses have confirmed truncated or size-reduced forms of the BRCA1 protein. The splice variants were not specific for the breast cancer patients analyzed.

Program Nr: 1751 from the 1999 ASHG Annual Meeting

Structural and functional evidence for the presence of a tumor suppressor gene in human non-small cell lung cancers on chromosomal region 11q23.2. *Y.S. Murakami[1], T. Nobukuni[1], Y. Sugiyama[1], M. Kuramochi[1], H. Fukuhara[1], T. Maruyama[1], T. Sekiya[1], M. Pletcher[2], R.H. Reeves[2]*. 1) Oncogene Div, Natl Cancer Ctr Research Inst, Tokyo, Japan; 2) Dept of Physiology, Johns Hopkins Univ. Schl. Med, Baltimore, MD.

Loss of heterozygosity (LOH) for the loci on chromosome 11q22-23 is observed at high frequency in human non-small cell lung cancers (NSCLCs). We recently reported that by spheroplast fusiong of YAC clones incorporating 1,100 kb of DNA from 11q23.2 into a human NSCLC cell line, A549, can completely suppress the tumorigenic phenotype of these cells. A nested deletion carrying 800kb of this YAC gives partial suppression. These findings provide functional evidence for the presence of a tumor suppressor gene(s) on 11q23.2. To identify this tumor suppressor gene, a contig map of the 1,100 kb region was constructed by screening human BAC and PAC libraries. Six polymorphic markers were mapped to high resolution on this contig. These markers were used to refine the localization of breakpoints in a subset of NSCLCs that showed LOH around this region. This allowed the commonly deleted NSCLC region to be narrowed to 1.0 Mb between the markers, 86CA and D11S1885. This region completely contains the entire 800 kb fragment with tumor suppresssor activity, providing independent confirmation of the presence of a tumor suppressor gene(s) in this region. To further refine the location of the tumor suppressor gene, PAC clones into A549 cells. The PAC vector includes the blasticidine-S-methylase gene, allowing selection of transfected cells with blasticidine. Resistant clones were screened by hybridization to detect the human sequences from the transferred PAC. Twelve transfected A549 cell lines were characterized by Southern blotting, demonstrating the presenece of one to ten copies of the insert DNA in various A549 cell lines. These lines are being tested to determine whether the included fragments suppress tumorigenicity of the parental A549 cells, providing a fucntional assay to localize this NSCLC tumor suppresor gene(s).

Program Nr: 1752 from the 1999 ASHG Annual Meeting

Cytogenetic analysis of two histopathological types of schistosome-related bladder tumors using comparative genomic hybridization. *M.T. Muscheck[1,2,4], H. Abol-Enein[3], K. Chew[1], P. Alken[2], P. Carroll[4], F. Waldman[1]*. 1) Cancer Genetics Program, UCSF Cancer Center, San Francisco, CA; 2) Department of Urology, Uni-Klinikum Mannheim gGmbH, Mannheim, Germany; 3) Urology & Nephrology Center, University of Mansoura, Mansoura, Egypt; 4) Department of Urology, UCSF, San Francisco, CA.

Schistosome-related bladder cancer shows different clinical and pathological features compared to non-schistosome-related bladder cancer. In this study, we used molecular cytogenetics to characterize regional differences in DNA sequence copy number in two histopathological types of schistosome-related bladder cancers from Egypt. Schistosome-related primary bladder tumors from 54 Egyptian patients with invasive disease (pT2-4) were analysed. Tumors were classified as squamous cell carcinomas (SCC; n=26) and transitional cell carcinomas (TCC; n=28. Genomic DNA was extracted after micro-dissection, amplified by DOP-PCR, followed by nick-translation and CGH. The mean number of alterations per specimen was significantly higher in TCC tumors (14.4) compared to SCC tumors (8.2, $p<0.001$). Most prevalent genetic alterations were 8p-, 8q+, and 18q- for both tumor types, 5q-, 9p-, 10q-, 11p-, and 11q- for TCC tumors, and 17p-, and 18p- for SCC tumors. After statistical correction of p-values, chi-square tests showed significantly different losses for chromosome arms 11p ($p=0.01$), 17p ($p=0.001$), 18p ($p=0.03$), and 21q ($p=0.03$). High level amplifications (green to red ratio > 1.5) of small chromosome regions were found in 15 TCC tumors and 8 SCC tumors. Amplification sites were 1q21-q23 (2), 6p22 (2), 8q21-q23 (3), 11q13 (3) for TCC tumors, and 1p21 (1), 1p31 (1), 8q22-q23 (2), and 11q13 (1) for SCC tumors. This study represents the first analysis of schistosome-related bladder cancer by CGH, and it confirms the presence of previously known aberrations and highlights new quantitative abnormalities and sequence amplifications. It strengthens the supposition that different tumor subgroups of the urinary bladder are characterized by distinct patterns of chromosomal alterations, which might explain differences in clinical behavior. Supported by: NCI CA 47537.

Program Nr: 1753 from the 1999 ASHG Annual Meeting

Loss of heterozygosity in different areas within high-grade diffuse astrocytoma. *A. Mutirangura[1], Sh. Shuangshoti[2], Y. Navalitloha[3], V. Kasantikul[2], S. Shuangshoti[2].* 1) Genetics Unit, Dept Anatomy, Fac Medicine, Chulalongkorn Univ, Bangkok, Thailand; 2) Department of Pathology, Fac Medicine, Chulalongkorn Univ, Bangkok, Thailand; 3) Department of Surgery, Fac Medicine, Chulalongkorn Univ, Bangkok, Thailand.

The primary objective of this study was to determine loss of heterozygosity (LOH) in various portions of 9 high-grade diffuse astrocytomas, 8 glioblastomas and 1 anaplastic astrocytoma. LOH was observed on chromosomes 9, 10, 17 and 19 in 8, 3, 4 and 2 cases, respectively. Genetic heterogeneity and a multistep process were identified in 4 glioblastomas explaining the diverse morphological characteristics, a common feature of diffuse astrocytomas. In 2, 2, 3 and 1 cases, the allele losses were found within part of grade IV astrocytomas but not grades II or III, on chromosomes 9, 10, 17 and 19, respectively. In one of these, while genetic heterogeneity was observed on chromosome 17 between the area of grade II and grade IV, 9pLOH was found within both areas and occurred on the same allele. The other 5 cases did not demonstrate genetic heterogeneity and the LOH was on the same allele, irrespective of grade, suggesting clonal origin. In conclusion, at the molecular level, the diverse morphological features of astrocytoma develops by a multistep mechanism of genetic alterations from one cell via low-grade and more malignant tumors towards glioblastoma.

Program Nr: 1754 from the 1999 ASHG Annual Meeting

Prostate cancer risk and repeat (CAG and GGN) polymorphism of the androgen receptor gene in an Australian population. *N.T Nassif, C. Kok, G.P Lobo, G.A Nicholson.* Molecular Medicine Laboratory, Department of Medicine, Sydney University, Clinical Sciences Building, Concord Hospital, Concord, NSW 2139, Australia.

Prostate cancer is the most commonly-diagnosed male malignancy and the second most common cause of male cancer death in the Western world. Androgens play an essential role in the growth of normal and malignant prostate cells. Androgen action within target cells is mediated by the androgen receptor (AR) which binds dihydrotestosterone and stimulates the transcription of androgen-responsive genes. Because of this association, it has been proposed that the AR might be one genetic predictor of an individual's susceptibility to prostate cancer. The transactivation activity of the AR resides in the amino-terminal domain of the protein encoded in exon 1 of the gene. Exon 1 contains in-frame polymorphic $(CAG)_{9\text{-}33}$ and $(GGN)_{8\text{-}17}$ trinucleotide repeats. The transactivation activity of the receptor has been shown to be inversely proportional to (CAG)n repeat length. Shorter CAG alleles cause a more active growth of prostate cells which, in turn, elevates the risk of prostate cancer since target cell proliferation is known to promote tumorigenesis. Our aim was to determine whether germline CAG and GGN repeat polymorphisms within the AR gene are associated with the development of prostate cancer. We have determined the CAG and GGN allele sizes of prostate cancer patients (n=360) and age-matched unaffected individuals (n=302). We have found that the prostate cancer patients had a mean CAG repeat size (21.68) that was smaller than that of the control (22.2) group (two sided $P<0.05$). No such difference in mean (GGN)n repeat number was observed between cases and controls. Further analyses revealed an increased risk in men with less than the median number of CAG repeats (<22) and who had an affected first degree relative. Men with shorter (n£16) GGN repeats also had elevated risk estimates. The subset of men with two short repeats (CAG<22 and GGN £16) had a 2-fold elevation in risk.

Program Nr: 1755 from the 1999 ASHG Annual Meeting

p53 mutation spectrum in non-small cell lung cancer: results from a prospective study. *H.H. Nelson[1], J.K. Wiencke[2], D.C. Christiani[1,3], E.J. Mark[3], J.C. Wain[3], K.T. Kelsey[1].* 1) Cancer Cell Biology, Occupational Health Program, Harvard School Public Health, Boston, MA; 2) Department of Epidemiology and Biostatistics, UCSF, San Francisco, CA; 3) Departments of Medicine, Pathology, and Surgery, Massachusettes General Hospital, Boston, MA.

p53 mutations were assessed in a large case-series of surgically resected lung tumors from a predominantly smoking population (n=354). The prevalence of mutation was 28%, and did not significantly differ by histology or gender. p53 mutations occurred with a similar frequency in current and former smokers (28%), and were less common in never smokers (18%). Previous studies, using meta-analysis and retrospective case designs, have established that transversion mutations (in particular G:C to T:A substitutions) are the dominant mutation event in lung tumors from smokers. Analysis of spectra in the current study revealed a strikingly low prevalence of G:C to T:A mutations (16.8%). C:G to T:A substitutions were the most common type of mutation (28.7%), with the majority of these occurring at non-CpG sites (70%). In addition, the prevalence of transitions, transversions, and specific base substitutions did not differ when the population was stratified on pack-years smoked. Finally, analysis of the distribution of mutations by codon did not reveal any hotspots. Comparison of our spectra data with the IARC database reveals significant differences in both the distribution of mutations along the gene, and the frequency of mutation types. These differences may reflect recent changes in the lung cancer epidemic.

Program Nr: 1756 from the 1999 ASHG Annual Meeting

Characterization of common BRCA1 and BRCA2 missense mutations. *S.L. Neuhausen[1], M. Hoffman[1], A. Deffenbaugh[2], S. Manley[2], T.S. Frank[2], B.E. Ward[2].* 1) Dept Medical Informatics, Univ Utah, Salt Lake City, UT; 2) Myriad Genetic Laboratories Inc, Salt Lake City, UT.

Over 1000 individual mutations have been identified in BRCA1 and BRCA2, genes responsible for inherited predisposition to breast and ovarian cancers. The functional significance, and therefore clinical significance, of many of the missense mutations has not been established. Some of these missense mutations have been observed repeatedly. No assay to test the function of missense mutations is available. Observations which suggest that a missense mutation is not causal are: 1) it is observed in a control group in equal or greater prevalence to an at-risk group; 2) it is not in a putative functional domain; 3) there is not cosegregation of the variant and disease in a family with significant disease; 4) the missense change results in replacement of a similar amino acid to the wild-type; and 5) there is not conservation of the wild-type amino acid between human, mouse, and dog BRCA1 or BRCA2 proteins.

The aim of this study was to characterize the clinical significance of 8 missense mutations that have been reported in multiple unrelated women who have undergone clinical testing for BRCA1 and BRCA2. Of the 8 mutations, none occurred in putative functional domains, 6 had dissimilar and 2 had similar amino changes, 5 were conserved and 3 were not conserved in human and mouse, and 6 were observed in individuals with a truncating mutation in the same gene. A control population, of 154 Caucasians of Northern European ancestry unaffected with cancer, was used to determine population frequencies of the mutations. Frequencies in the control population ranged from 0% to 3.3%. Based on the criteria described above, the BRCA1 S1512I and M1652I and BRCA2 A2951T mutations are polymorphisms, the BRCA1 R1347G and BRCA2 D1420Y and V2728I mutations are likely polymorphisms, and the BRCA2 T598A and R2034C mutations remain missense mutations of unknown significance. Additional control samples are being examined for the more rare mutations, such as T598A. Cosegregation of mutations within families also needs further exploration.

Program Nr: 1757 from the 1999 ASHG Annual Meeting

Expression analysis of candidate endometrial tumor suppressor genes from 10q25.3. *F.C. Noonan[1], D.G. Mutch[2], P.J. Goodfellow[1]*. 1) Surgery, Washington University, St. Louis, MO; 2) Obstetrics and Gynecology, Washington University, St. Louis, MO.

LOH studies in endometrial tumors have mapped a tumor suppressor gene to a minimum deletion interval of 300 kb on chromosome 10q25.3. Genomic sequence for the region has facilitated our sequence-based approach to candidate gene isolation. We have identified multiple cDNAs in the region by EST mapping. Northern and dot blot analysis undertaken to prioritize candidates has revealed four apparantly independent transcripts with expression patterns consistent with a potential role in tumorigenesis. All four candidates are expressed highly in normal uterus. We have begun to isolate full length cDNAs corresponding to these ESTs. The first candidate, F1, is expressed in three tissues: uterus, kidney and brain, and detects a transcript of 3 kb. F1 shows evolutionary conservation by Southern hybridization to rodent genomic DNA and sequence homology to two mouse ESTs [88% identity over 258 bp]. In addition, F1 contains part of the coding sequence of the second candidate, the homeobox gene *EMX2*. *EMX2* detects a ubiquitous 5 kb transcript, and an abundant 3 kb transcript in the uterus. We are attempting to clarify the relationship between these two transcripts by isolating both uterus and kidney cDNAs. The third candidate, F5C, has an expression pattern similar to F1; it is expressed highly in the uterus, kidney and brain. However, F5C detects an 8 kb transcript. Screening three cDNA libraries has yielded four clones, the longest of which is only 1 kb. RACE is being used to isolate a more complete sequence for this large transcript. The fourth transcript, F4 is expressed only in the uterus. The localization of these genes in a relatively small genomic interval and the expression patterns suggests that this may be a gene complex involved in uterine development and/or morphogenesis. Sequence analysis of the partial cDNAs (with the exception of *EMX2*) indicates that all correspond to novel genes. *In situ* hybridization analysis has been initiated in endometrial tissues at different stages of proliferation to learn more about the temporal and spatial expression patterns of these transcripts.

Program Nr: 1758 from the 1999 ASHG Annual Meeting

t(8;12) Induces Aberrant *HMGIC* Expression In Aggressive Angiomyxoma Of The Vulva. *M.R. Nucci[1,2,4], S. Weremowicz[2,4], G. Tallini[5], K. Sornberger[2], C.C. Morton[2,3,4], B.J. Quade[1,2,4].* 1) Div. of Women's and Perinatal Pathology; 2) Dept. of Pathology, and; 3) Dept. of OB/GYN, Brigham and Women's Hospital, Boston, MA; 4) Harvard Medical School, Boston, MA; 5) Dept. of Pathology, Yale Medical School, New Haven, CT.

Background: Benign mesenchymal tumors associated with rearrangements of *HMGIC*, a DNA architectural factor gene mapping to 12q15, include cutaneous lipomas, uterine leiomyomas, pulmonary chondroid hamartomas, endometrial polyps, pleomorphic adenomas of the salivary gland, and breast fibroadenomas and hamartomas. Fusion gene formation predominates in lipomas and pleiomorphic adenomas. In contrast, rearrangements in leiomyomas result in *HMGIC* overexpression. Although *HMGIC* has also been implicated in the pathobiology of vulvar aggressive angiomyxoma (VAA), the molecular mechanism(s) pertaining to this neoplasm are unclear. **Design:** Tissue from a recurrent VAA was used for cytogenetic and FISH analysis and for RT-PCR or immunohistochemical analysis of *HMGIC*, HMGI(Y), or *G3PDH* expression. **Results:** The GTG-banded karyotype was 46,XX,t(8;12)(p12;q15). FISH with whole chromosome painting probes excluded additional occult rearrangement. The chromosome 12 breakpoint was mapped with two-color FISH using cosmid probes 5' (centromeric) and 3' (telomeric) to *HMGIC*. Signals for both cosmid probes showed hybridization on the normal chromosome 12 and der(12) chromosome, indicating that the breakpoint was 3' (telomeric) to the gene. RT-PCR revealed *HMGIC* expression in the tumor. Immunohistochemistry localized HMGIC expression to the tumor's spindle cells, but not to blood vessels. HMGI(Y) expression was not detected by immunostaining. **Conclusions:** Like numerous benign mesenchymal tumors, this locally aggressive tumor is associated with rearrangements of *HMGIC*. Our results show that chimeric gene formation is not required for tumorigenesis. Inappropriate expression of this DNA binding protein in the tumor's spindle cell component, however, is important in this tumor's pathobiology. Understanding the pathogenetic mechanism in VAA may also provide new insights into invasiveness in other tumors and in management of residual disease in VAA.

Program Nr: 1759 from the 1999 ASHG Annual Meeting

Cytogenetic and molecular genetic analysis of renal tumors in Singapore. *P.L. Ong[1], P.H. Tan[1], W.O. Lui[2], L.C. Lau[1], Y.Y. Chong[1], T.W. Chong[3], K.T. Foo[3], S.L. Tien[1]*. 1) Dept of Pathology, Cytogenetics Laboratory, Singapore General Hospital, Singapore; 2) Dept of Medical Oncology, Singapore General Hospital, Singapore; 3) Dept of Urology, Singapore General Hospital, Singapore.

In our study of 17 renal tumors in Singapore, 12 were clear cell RCC, 2 were papillary RCC, 1 had both clear cell and papillary foci and 2 were transitional cell carcinoma (TCC) of the renal pelvis. The patient cohort comprised 13 males and 4 females ranging from 44 to 73 years old. 13 were Chinese and 4 were Malay in ethnic origin. 10 clear cell RCCs had chromosome 3 aberrations; the eleventh had -8 while the twelfth had +21. 6 had -3 and 3 had t(3;5). The papillary RCCs had gains or losses of whole chromosomes. The TCCs had +3, -9 and -21 in common, one of which had a novel add(3)(q27). The retinoblastoma susceptibility 1 (RB1) gene at 13q14 was also deleted in this tumor. DNA was extracted from the renal tumors of these patients and PCR performed at the VHL locus. We identified 3 novel frameshift mutations in 3 clear cell RCCs. The mutations, 587delAC and 628insA in exon 2, and 691delGA in exon 3, result in stop codons downstream from the mutation site and will produce truncated proteins. On cytogenetic analysis, the structural integrity of the VHL gene of the 3 tumors with mutations characterized was maintained, suggesting that the karyotypes underestimated the extent of genetic loss. In conclusion, we have identified chromosomal aberrations in our 17 patients with renal tumors, some which have not been previously documented. This may indicate the emergence of new anomalies in patients of Asian origin. In addition, cytogenetic analysis, complemented with molecular techniques, will enhance the detection of genetic aberrations in renal tumors. Furthermore, mutation searches of proto-oncogenes or tumor suppressor genes known to be involved in RCC should be done to fully understand the genetics of such tumors.

Program Nr: 1760 from the 1999 ASHG Annual Meeting

Gene Expression Profiles of Ovarian Cancer Using T7 based RNA Amplification and cDNA Microarray Technology. *K. Ono[1], T. Tanaka[1], O. Kitahara[1], N. Shiraishi[1], C. Kihara[1], T. Seki[1], R. Yanagawa[1], H. Ogasawara[1], T. Okamoto[2], Y. Ohnishi[3], K. Kuzuya[4], T. Tsunoda[5], T. Takagi[5], Y. Nakamura[1]*. 1) Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science University of Tokyo; 2) Dept.of Obstet. and Gynecol., Nagoya Univ. Sch. of Med; 3) Dept.of Obstet. and Gynecol., Kagoshima City Hospital; 4) Dept.of Gynecol., Aichi Cancer Center; 5) Laboratory of Genome Database, Human Genome Center, Institute of Medical Science, University of Tokyo.

The microarray hybridization technology enabled us to study the expression of a large number of genes simultaneously. However, since the RNA expression analysis of cells using the microarray requires a few micrograms of mRNA, we applied T7-based RNA amplification method to obtain sufficient quantities of RNA from limited materials. Total RNAs were isolated from fresh-frozen normal and tumor specimens from patients with ovarian cancer. Following RNA amplification with T7 RNA polymerase, we labeled cDNA probes by Cyanine dyes and hybridized to the microarrayed cDNAs of 4476 genes. Probes amplified from total RNA and those prepared from mRNA from the same material revealed a very similar expression profile, indicating that the limited quantities of RNA (from 1000 or more cells) can be used for microarray analysis. Furthermore, the microarray analysis showed that expression patterns of several genes in ovarian cancer were correlated with the features of pathological or clinical classification of the samples. Thus, cDNA microarray technology in combination with T7-based RNA amplification method will provide the useful information for the identification of genes involving in tumorgenesis as well as molecular targets for diagnosis and therapeutic intervention .

Program Nr: 1761 from the 1999 ASHG Annual Meeting

Cytochrome P450c17a **gene(CYP17) polymorphism is associated with risk for colorectal adenomas.** *E.C. Osth[1], R. Haile[1], H-J. Lenz[2], E.R. Lee[3], H. Frankel[4], G. Coetzee[5], S.A. Ingles[1]*. 1) Dept. of Preventative Medicine, University of Southern CA, Los Angeles, CA; 2) Dept. of Hematology and Oncology, University of Southern CA, Los Angeles, CA; 3) Division of Gastroenterology, Sunset Kaiser Permanente Medical Center, Los Angeles, CA; 4) Division of Gastroenterology Bellflower Kaiser Permanente Medical Center, Los Angeles, CA; 5) Dept. of Microbiology, University of Southern CA, Los Angeles, CA.

Recent findings on hormone replacement therapy (HRT), genetic control of estrogen levels, and age and sex differences in the incidence of colorectal cancer have motivated our hypothesis that genetically controlled variation in endogenous estrogen levels might influence risk of colorectal cancer in women. Epidemiologic data have shown that HRT is protective against colon cancer in women and polymorphism in the CYP17 gene has been associated with serum estradiol levels and with age of menarche. We conducted a case-control study of 230 colorectal adenoma cases and 241 controls. All subjects were members of a prepaid health plan in Los Angeles who underwent sigmoidoscopy in 1991-1993. We found that women carrying at least one copy of the CYP17 A2 allele were at increased risk of colorectal adenomas. Compared to women with the A1/A1 genotype, the odds ratios were 1.33 (CI: 0.80, 2.02) for women with genotype A1/A2 and 2.18 (CI: 1.19, 4.00) for women with genotype A2/A2. Issues regarding our results and findings that associate HRT and colorectal cancer will be discussed.

Program Nr: 1762 from the 1999 ASHG Annual Meeting

Identification of genes overexpressed in medulloblastoma by SAGE. *E. Oussoren[1], O. Wirths[2], A.L.M.A. ten Asbroek[1], E. Pauws[1], T. Pietsch[2], F. Baas[1].* 1) Neurozintuigen, Academic Medical Center, Amsterdam, Netherlands; 2) Department of Neuropathology, University of Bonn Medical Center, Bonn, Germany.

Medulloblastoma is a malignant primitive neuroectodermal tumor of the central nervous system, occurring predominantly in childhood and arising in the cerebellum. Five-year survival of these children is still poor (39-70%) despite very intensive therapy consisting of neurosurgery, radiotherapy and sometimes chemotherapy. SAGE was used to search for genes that play a role in development and growth of medulloblastomas. The gene expression pattern of a 24 week fetal brain was compared with that of a medulloblastoma. Here we report the identification of two tags (#17 and 18) which were significantly higher expressed in medulloblastoma compared to fetal brain. Tag #17,which was found 13/10,000 SAGE tags in a classic medulloblastoma, was expressed in all medulloblastomas (n=10) tested thus far. Tag #17 matched with multiple ESTs. Sequence analysis of new clones identified with this tag shows homology with the family of the IGF binding proteins. Tag #18 matched to OTX2, the human homolog of the Drosophila gene orthodenticle. Expression patterns of OTX2 were determined by Northern blot and semi-quantitative PCR. In total 42 medulloblastomas were analyzed (32 of classic, 10 of desmoplastic histology). 26 of the classic medulloblastomas but only 2 of desmoplastic tumors showed high OTX2 mRNA, whereas adult tissues, fetal and adult cerebellum had no or very low mRNA levels. In conclusion, two genes highly expresssed in medulloblastoma were identified by SAGE. Both are expressed in the majority of the medulloblastomas tested thus far and may serve as molecular markers for these malignant tumors.

Program Nr: 1763 from the 1999 ASHG Annual Meeting

A hospital-based study to determine the genetic basis of non-medullary thyroid cancer. *T. Pal[1,2], R. Tsang[3], J. Brierley[3], S.A. Narod[2]*. 1) Familial Cancer Clinic, Princess Margaret Hosp, Toronto, ON, Canada; 2) The Centre for Research in Women's Health, University of Toronto, ON, Canada; 3) Department of Radiation Oncology, Princess Margaret Hosp, Toronto, ON, Canada.

The non-medullary forms of thyroid cancer are not generally thought of as having a familial basis; however, epidemiologic and family studies suggest that a subset of these cancers may be due to an inherited predisposition. In order to determine how frequently non-medullary thyroid cancer is due to a hereditary factor, we conducted a hospital-based study at the Princess Margaret Hospital, Toronto, Canada. We obtained pedigrees on 184 patients diagnosed with non-medullary thyroid cancer . Family histories of cancer were obtained for all 1339 first-degree relatives of these patients; in addition, family history of thyroid cancer was obtained on second and third degree relatives . There were 13 families (7%) with 2 or more cases of non-medullary thyroid cancer in either first, second or third degree relatives. The observed cancer rate in the relatives was compared with the expected number, based on comparison with age-standardized provincial incidence rates in order to estimate relative risks. For thyroid cancer, there were 11 cancers observed in relatives, compared to the 3.0 cancers expected, giving a relative risk of 3.7 (95% confidence interval (CI): 1.9-6.7). Our findings suggest that a small proportion of non-medullary thyroid cancer has an inherited basis. Further molecular studies are needed to determine the genetic basis of cancer in these families.

Program Nr: 1764 from the 1999 ASHG Annual Meeting

Mutation detection in BRCA1 using real time PCR and melting curve analyses. *G. Pals[1], K. Pindolia[2], M.J. Worsham[2]*. 1) Inst Human Genetics, Free Univ, Amsterdam, Netherlands; 2) Cancer Genetics Research, Pathology, Henry Ford Health Systems, Detroit, MI.

The detection of two recurrent mutations in the hereditary breast/ovarian cancer associated BRCA1 gene was studied with real time PCR and melting curve analyses. Short PCR products were designed around the small deletion 185delAG in exon 2 (recurrent in the Ashkenazi Jewish population) and the single base substitution 2841GT in exon 11 (recurrent in the Dutch population). Hybridization probe sets were designed for both PCR products, with each probe overlapping the specific mutation. The exon 11 probe set also covered two other mutations, the 2809insA and the 2814insA also recurrent in the Dutch population. The 3'end of the 5' probe was labeled with FITC and the 5'end of the 3' probe with LightcyclerRed640. The 185del and the 2841G mutations were easily detected with the hybridization probes, resulting in dual peaks for heterozygotes in melting curve analyses. The differences in melting characteristics of the heteroduplexes in heterozygotes were not detectable with SYBRgreen. Not all mutations covered by the probes could be detected, indicating that the method is not suitable for unknown mutations. For known mutations, melting curve analysis using hybridization probes is highly sensitive, rapid and an efficient approach to mutation detection.

Program Nr: 1765 from the 1999 ASHG Annual Meeting

Identification and characterization of genes differentially expressed in a subset of Wilms tumors with non-functional WT1. *H. Pannu, V. Huff.* Department of Experimental Pediatrics, University of Texas MD Anderson Cancer Center, Houston, TX.

Wilms tumor is a childhood renal malignancy that occurs in both sporadic and familial forms. The disease is genetically heterogeneous, and studies have implicated loci at 11p13, 11p15, 1p, 7p, 16q, 17q and 19q in the development of Wilms tumor. Of these, the transcription factor WT1 at 11p13 is the only Wilms tumor gene cloned and characterized to date. To identify other cellular genes that are potentially important in the development of Wilms tumor, we performed differential display analysis, comparing gene expression in a panel of six Wilms tumors which had sustained inactivating mutations at the WT1 locus with two other childhood renal tumors, normal kidney, and fetal kidney. Using a set of 104 primer combinations for differential display RT-PCR, we identified sixteen bands representing genes that are differentially expressed in Wilms tumors as compared to control tissues. We repeated the analysis in an independent RT-PCR amplification using the primer sets that generated these differentially expressed bands and were able to confirm differential expression for eleven of these gene fragments. We used these eleven gene fragments to probe Northern blots and determined that three of these detect transcripts that are differentially expressed in Wilms tumors as compared to controls. These three have been subcloned and sequenced. We have used the initial sequence (~250-550 bp) to screen available sequence databases and have identified a few highly homologous ESTs. We have screened a fetal kidney cDNA library for full length clones and will determine the chromosomal location of these genes using a radiation hybrid panel. In a further effort to define the role of these genes in Wilms tumor, we are currently analyzing the expression of these genes in genetically defined subgroups of Wilms tumor and in a range of normal fetal and adult tissues.

Program Nr: 1766 from the 1999 ASHG Annual Meeting

Characterization of chromosomal aberrations in six Korean gastric carcinoma cell lines using chromosome painting. *S.H. Park, J.I. Kil, S.Y. Park, H. Kim, Y.H. Chun.* Institution of Human Genetics, Dept. of Anatomy, Korea University College of Medicine and Gradute School of Biotechnology, Korea University, Seoul, Korea.

Gastric cancer is the most prevalent form of cancer in east Asia, including Korea and Japan. It is difficult to culture the stomach cancer cells and to analyze the chromosome aberrations. The purpose of this study was to establish in detail karyotypes of Korean gastric cancer cell lines, using multiple chromosome paintings. Six Korea gastric carcinoma cell lines(SNU-484, SNU-601, SNU-620, SNU-638, SNU-668, SNU-719) were cultured and harvested for cytogenetic analysis. Routine karyotyping was performed. For complete analysis of chromosomal aberrations, human chromosome-specific painting probes were constructed from somatic hybrid cell DNAs and region specific painting probes were made by amplification of microdissected chromosome bands. The origins of the unidentified marker chromosomes were analyzed by FISH with these painting probes. Each cell lines had unique modal kayotypic characteristics and showed a variable number of numerical and structural clonal cytogenetic aberrations. SNU-484, -620, -668 had near triploidy and SNU-601, -638, -719 had near diploidy, and the number of marker chromosome of SNU-484, -620, -668, -601, -638, -719 was 13-15, 8, 25-27, 15-20, 6, 3-6, respectively. The origins of the marker chromosomes of these cell lines were identified by FISH with constructed painting probes. Especially rearrangement of chromosome 17 resulting in partial deletion of 17p(and/or partial gain of 17q) and centromere involved translocations were found in all six cell lines. The most frequent marker observed in these cell lines was a partial gain or deletion of chromosome 7 with the breakpoints in regions 7q22-q32.

Program Nr: 1767 from the 1999 ASHG Annual Meeting

NO EVIDENCE OF CORRELATION BETWEEN P53 CODON 72 POLYMORPHISM AND RISK OF HPV-16 ASSOCIATED CERVICAL CARCINOMAS IN KOREAN WOMEN. *Y.S. Park, M.Y. Kim, H.J. Lee, M.J. Oh, K.O. Lee.* Molecular Diagnosis, Seoul Medical Science Institute, SCL, Seoul, Korea.

Human papillomavirus (HPV)-16 and 18 encode E6 oncoprotein, which binds to and induces degradation of the cellular tumor suppressor protein p53. A common polymorphism of p53, encoding either proline or arginine at position 72, affects the susceptibility of p53 to E6-mediated degradation. A recent report suggested that the risk of HPV associated cervical carcinomas in Caucasian women is about seven time higher for those homozygous for the arginine allele than for those who are heterozygous. To examine whether arginine 72 could be a risk factor for HPV associated cervical carcinomas in the Korean population, we used the PCR-RFLP method to analyze p53 polymorphism of HPV-16 positive cervical carcinomas from 109 Korean women versus 111 control samples. The proportions of individuals homozygous for the arginine allele, homozygous for the proline allele, and heterozygous for the two alleles were 31%, 17%, and 52% among the control group; and 38%, 11%, and 51% in women with HPV-16 positive cervical carcinomas. As we detected no significant difference in the frequencies of proline or arginine alleles between the two groups, p53 polymorphism at residue 72 does not seem to be involved in the development of HPV associated cervical carcinomas in women of Korean ethnicity.

Program Nr: 1768 from the 1999 ASHG Annual Meeting

Spinal magnetic resonance (MR) imaging findings in neurofibromatosis 2 (NF2) and correlations with *NF2* germline mutations. *D.M. Parry[1], C.M. Bromley[1], N. Courcoutsakis[2], G.L. Katzman[2], M. MacCollin[3], N. Patronas[2].* 1) Genetic Epidemiology Br, National Cancer Inst, Bethesda, MD; 2) Clinical Center, National Institutes of Health, Bethesda, MD; 3) Massachusetts General Hospital, Boston, MA.

Neurofibromatosis 2 (NF2) is an autosomal dominant disorder characterized by bilateral vestibular schwannomas, other central and peripheral schwannomas, meningiomas and cataracts. Spinal tumors have been seen in 75-89% of patients who have had MR imaging of the entire spine. Based on histopathology, extramedullary tumors are usually schwannomas or meningiomas, and intramedullary tumors are usually ependymomas. The *NF2* gene has been cloned from 22q and germline mutations have been identified in many NF2 patients. Comparisons of genotypes with phenotypes suggest that, in general, nonsense and frameshift mutations are associated with earlier ages at onset and diagnosis, having symptoms before age 20, and higher percentages with and higher numbers of intracranial meningiomas, spinal tumors and skin tumors. The different types of spinal tumors have not been examined in conjunction with genotype. We reviewed spinal MR images from 49 NF2 patients from 26 unrelated families. The tumors were counted and extramedullary tumors were classified as being either nerve sheath tumors (NSTs) or meningiomas. Intramedullary, extramedullary, and both types of tumors occurred, respectively, in 53%, 55% and 45% of the patients. Genotypes were known for 37 patients from 19 families: 10 families had nonsense or frameshift mutations, 7 had splice site mutations, and a missense mutation and an in-frame deletion were each present in a single family. Statistical analysis that adjusted for intra-family correlation demonstrated that a higher proportion of patients with nonsense or frameshift mutations had intramedullary tumors ($P<.025$) compared to the group of patients with all other types of mutations. In unadjusted analyses, patients with nonsense or frameshift mutations also had higher mean numbers of intramedullary tumors ($P<.006$) and NSTs ($P<.0001$). These results suggest that the association between nonsense and frameshift mutations and severe NF2 may extend to specific categories of spinal tumors.

Program Nr: 1769 from the 1999 ASHG Annual Meeting

Analysis of the *TSC1* and *TSC2* Genes in Sporadic Glial and Glioneuronal Tumours. *L. Parry[1], J. Maynard[1], S.A. Price[1], A. Patel[1], S. Whittaker-Axon[1], A. Von Diemling[2], J.P. Cheadle[1], J.R. Sampson[1]*. 1) Institute of Medical Genetics, University of Wales College of Medicine, Cardiff, Wales, UK, CF4 4XN; 2) Institute of Neuropathology, Berlin.

We studied paired constitutional and tumour DNA samples from 91 patients with sporadic glial and glioneuronal tumours for loss of heterozygosity at the TSC loci using a combination of 7 previously reported and 7 novel polymorphic markers. A relatively high frequency of LOH was seen in gangliogliomas (2/7 tumours informative for *TSC1* markers and 3/11 tumours informative for *TSC2* markers). Lower levels of LOH were seen in other types of brain tumour - 1/16 astrocytomas informative at both loci and 4/15 ependyomas, 2/14 glioblastoma multiforme, 0/7 oligodendromas, 0/7 tumours of mixed oligodendrocytic/astrocytic histology and 2/11 pilocytic astrocytomas.

An SSCP exon screen of the *TSC1* gene on the tumours displaying LOH at that loci revealed no mutations within the conserved allele. Tumours displaying *TSC2* LOH are currently being SSCP exon screened for the gene. All gangliogliomas are being SSCP screened for all exons of the TSC genes.

Previous non-targeted LOH studies on these tumours revealed no LOH in the gangliogliomas. This higher incidence of TSC LOH in these tumours which are histologically the most similar to atypical tuberous sclerosis hamartomas of the brain, make further molecular studies important to assess any role the TSC genes may play in the development of these sporadic gangliogliomas.

Program Nr: 1770 from the 1999 ASHG Annual Meeting

THREE NOVEL MUTATIONS IN THE NF2 TUMOR SUPPRESSOR GENE. *C. Paz-y-Mino[1,2], J.C. Pérez[1], R. Burgos[1], V. Dávalos[1], M. Pérez[3], E. Hermida[4], P.E. Leone[1,2].* 1) Hum Mol Genet & Cytogen Lab. , Ecuadorian Catholic University, Quito, Ecuador; 2) Medicine Faculty, Ecuadorian Catholic University; 3) E. Espejo Hospital; 4) Social Security Hospital.

Neurofibromatosis Type 2 (NF2) is a dominantly inherited disorder characterized by a predisposition to multiple tumors of the central nervous system, particularly schwannomas and meningiomas. The NF2 tumor suppressor gene has been assigned to chromosome 22. Cytogenetic and LOH studies have suggested that the gene responsible for the development of schwannomas and meningiomas is located on chromosome 22q, and it has been proposed that some sporadic schwannomas and meningiomas and NF2 associated tumors may result from functional inactivation of the NF2 gene. We analyzed 57 tumors of the central nervous system for mutations in the NF2 and single-strand conformational polymorphism (SSCP) analysis was used to identify six tumors with an altered NF2 gene in four of the ten exons of the NF2 gene studied exons (2, 4, 5, 7, 8, 9, 10, 11, 12 and 15). Samples displaying mobility shifts were subjected to DNA sequencing. We describe three novel mutations, 2 in meningiomas and 1 in schwannomas. Mutations involved exons 9 and 12, and correspond to one missense, one splice acceptor and one polymorphism. This study is supported by the Project BID-111.

Program Nr: 1771 from the 1999 ASHG Annual Meeting

***MEST* (7q32) is imprinted in human breast tissue and biallelically expressed in infiltrating carcinomas of the breast.** *I.S. Pedersen[1], P.A. Dervan[1,2], D. Broderick[1], M. Harrison[2], N. Miller[3], E. Delany[1], D. O'Shea[1], A. McGoldrick[1], G. Keating[2], B. Tobin[2], A. McCann[1]*. 1) Department of Pathology, Biotechnology Centre, Lab.1, Dublin 4, Ireland; 2) Department of Histopathology, Mater Hospital, Dublin 7, Ireland; 3) National Centre for Medical Genetics, OLHSC, Crumlin, Dublin 12, Ireland.

Genomic imprinting refers to the mechanism by which selected genes throughout the genome are monoallelically expressed according to their parental origin. The paternally expressed gene *MEST* is the first imprinted gene to be identified on chromosome 7. The *MEST* gene is located in a chromosomal region harboring genetic alterations in a high proportion of invasive breast carcinomas. Altered imprinting is known to be involved in the progression of both embryonal and sporadic tumors. The aim of this study was to investigate the imprinting status of *MEST* in a series of 53 paired normal and breast tumor samples. Heterozygosity and allelic-usage was assessed using a transcribed *Afl*III restriction fragment length polymorphism. Of the 53 patients, 12 (23%) were informative. Only 1 tumor (a ductal carcinoma *in situ*) showed loss of heterozygosity (LOH). RNA analysis established that the lost allele was the inactive copy. Since LOH often is accompanied by a duplication of the retained allele, it is possible that the outcome of LOH in this case is equivalent to the outcome of LOI - 2 active gene copies. *MEST* RT-PCR products from 8 of the 11 patients with retained heterozygosity were analyzable. Seven of the tumor samples showed biallelic expression, whereas a functional imprint, evident by monoallelic expression, was in place in the adjacent normal tissue. All biallelic cases were infiltrating carcinomas. One sample (a case of atypical ductal hyperplasia) had maintained imprinting. Epigenetic changes in the form of re-activation of a normally silent/imprinted allele is a well-established mechanism for overexpression of growth-promoting genes in cancer. This is, however, the first study to show altered imprinting of *MEST* in cancer.

Program Nr: 1772 from the 1999 ASHG Annual Meeting

Mapping the Papillary Renal Cell Carcinoma Gene Between Loci D17S787 and D17S1799 on Chromosome 17q21.32. *T. Pesti[1], I. Balint[1], J. Fischer[1], B. Ljungberg[2], G. Kovacs[1].* 1) Dept. of Urology, Ruprecht-Karls-University, Heidelberg, Germany; 2) Dept. of Urology, University of Umea, Sweden.

Chromosome 17q is frequently duplicated in hereditary (HPRCT) and sporadic papillary renal cell tumors (RCT) and considered to be the initial genetic change. To determine the gene region we have first analysed 37 papillary RCTs by applying 25 polymorphic microsatellite markers and found an overlapping duplication including loci D17S1795 and D17S1306 on chromosome 17q21. This region was than analysed in additional 118 sporadic and hereditary papillary RCTs. Overlapping duplications at the chromosome 17q21.32 region localized the papillary RCT gene to an approximately 300 kb genomic sequences flanked by loci D17S787 and D17S1799. We found allelic duplication at this region in 100 of 112 sporadic and 42 of 43 hereditary cancers including small lesions of 2 mm in diameter. Altogether, 92% of the 155 papillary RCT showed allelic duplication at this region. We have included multiple papillary RCTs from three different individuals having no familial records or germ line mutation of the MET and from three families with HPRCT, two of them showing a germ line mutation of the MET. Duplication of both parental alleles in multiple tumors indicates that allelic changes at this region are somatic events. The hepatic leukemic factor (HLF) is localized within this region. In acute pro-B-leukemia showing the t(17;19) the E2A-HLF fusion gene inhibit the apoptosis suggesting that the chimeric protein contributes to leukemic transformation. We have analyzed the expression of HLF in distinct types of RCT by RT-PCR and did not find any changes that seems to be specific for papillary RCT. Therefore, alteration of another gene from the 300 kb region is responsible for development of hereditary and sporadic RCTs.

Program Nr: 1773 from the 1999 ASHG Annual Meeting

***APC* mutation in woman with multiple desmoids: A new syndrome or FAP variation?** *J.A. Peters[1,2], M.T. Lotze[2], L. Sproul[3], V. Pratt[4], W.S. Rubinstein[1,2]*. 1) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, PA; 2) University of Pittsburgh Cancer Institute; 3) Carlow College, Pittsburgh, PA; 4) Labcorp, Research Triangle Park, NC.

Desmoid tumors are proliferative, locally invasive, non-metastasizing fibromatous tumors resistant to treatment. Desmoids may occur sporadically, or as part of Familial Adenomatous Polyposis in about 10% of FAP families. We evaluated a 31 year old woman with multiple desmoid tumors who underwent initial resection at age 18 years. She had multiple additional tumors removed from her right back and neck, abdomen, and left flank. Surgery and chemotherapy failed to arrest tumor progression. Additional medical problems included seizures since infancy, becoming grand mal at puberty; protruding mandible and one supernumerary tooth; psoriasis; and probable mental delay, anxiety, depression, poor impulse control, immaturity, inability to live independently, and unusual personality and speech pattern. The patient has no history of colon polyps or tumors as evaluated by colonscopy. The family history is negative for desmoids, polyps, and colon tumors, but positive for possible mental illness in the paternal history. She has a son with developmental delay and a variety of behavioral problems, currently diagnosed as Tourette syndrome. Cytogenetic studies revealed that both mother and son have normal karyotypes. On molecular testing, our patient had a truncating APC mutation near codon 1694. APC studies on her son are pending. A frameshift APC mutation at codon 1924 has been reported in another family with multiple desmoids but minimal polyposis (Eccles et al., 1996, AJHG 59:1193-1201).

Our case raises a number of questions: How broad is FAP expressivity? Does this patient have an FAP variant such as Gardner syndrome in the absence of all colon symptoms and signs? Can the neuropsychiatric findings be accounted for on the basis of the APC mutation? What proportion of desmoid patients have a germline APC alteration? Does an APC mutation in this family predispose only to desmoids or also to polyposis? Do modifier genes play a role in phenotypic expression of APC alterations?

Program Nr: 1774 from the 1999 ASHG Annual Meeting

Familial aggregation of cancer at multiple sites in families of Ashkenazic Jewish non-carriers of the *BRCA1* mutation 185delAG. *L.E. Peterson[1], L.C. Friedman[2], S.E. Plon[3].* 1) Dept Medicine; 2) Dept Psychiatry & Behav. Sci; 3) Dept Pediatrics, Dept Mol. & Hum. Genet., Baylor College of Medicine, Houston, TX.

The goal of this investigation was to study familial aggregation of cancer at multiple sites in families of 244 (27 males, 217 females) Ashkenazi Jewish (AJ) counselands who tested negative for the *BRCA1* 185delAG mutation in a community-based study. The presence of familial aggregation of cancer at multiple sites among non-carrier AJ families may provide insight into genes that predispose to a variety of cancers. Reported histories of cancer identified 43 unconfirmed cases among female counselands (29 breast, 2-ovarian, 2-colon, 2-skin, 8-other), 72 male and 130 female unconfirmed cases among 1,322 first-degree relatives (624 males, 698 females), and 133 male and 191 female unconfirmed cases among 2,135 (1,085 males, 1,050 females) second-degree relatives (3,457 total). Poisson regression was used to test the hypothesis of equal cancer risk in first-and second-degree relatives, assuming independence of risk between relatives. For males, the relative risk (*RR*) of cancer among first-degree relatives (compared with second-degree relatives) was 2.87(95% CI, 2.13-3.86) for all cancers, 1.63(0.43-6.24) for stomach, 2.51(1.28-4.93) for colorectal, 2.90(0.95-8.90) for pancreatic, 2.92(1.28-6.67) for lung, 6.86(1.61-29.22) for skin, 5.00(2.63-9.51) for prostate, 2.81(0.18-44.88) for testicular, 5.54(1.32-23.26) for bladder, 2.90(0.92-9.12) for kidney, 2.13(0.21-21.86) for brain, 1.44(0.26-7.88) for Hodgkin's disease, and 1.47(0.29-7.50) for leukemia. Among females, *RR* was 3.61(2.89-4.50) for all cancers, 0.35(0.05-2.72) for stomach, 2.10(0.96-4.60) for colorectal, 1.82(0.36-9.29) for liver, 0.50(0.06-4.22) for pancreatic, 3.14(1.16-8.49) for lung, 21.65(2.62-178.56) for skin, 3.92(2.86-5.37) for breast, 2.05(0.40-10.43) for uterine, 8.84(3.46-22.59) for ovarian, 5.88(0.93-37.39) for bladder, 1.87(0.16-22.10) for kidney, 2.73(0.68-10.92) for Hodgkin's disease, and 2.10(0.35-12.67) for leukemia. Aside from possible sources of bias, cancer at sites for which *RR*>1 may be causally related to susceptibility genes expressed at multiple sites. (Supported by KO7-CA78199-01).

Program Nr: 1775 from the 1999 ASHG Annual Meeting

Genetic Counseling and Testing for Hereditary Breast/Ovarian Cancer: The Impact of Receiving Positive and Uninformative Results. *S.K. Peterson[1], E.R. Gritz[1], S.W. Vernon[2], S.K. Marani[1], P.T. Rieger[1], P.A. Ward[1], G.B. Mills[1]*. 1) UT MD Anderson Cancer Center, Houston, TX; 2) UT School of Public Health, Houston, TX.

We report data from an ongoing study regarding psychosocial aspects of genetic counseling and testing for hereditary breast and ovarian cancer (HBOC) in a clinical setting. Fifty two high-risk women (23 of whom had a prior breast or ovarian cancer diagnosis) underwent genetic counseling and testing for HBOC, and completed psychosocial measures before pre-test genetic counseling (baseline) and within 2 weeks of receiving their genetic test results. We compared differences in scores on these measures, from baseline to 2-week follow-up, among women who received positive (BRCA1/2 mutation was found) or uninformative (no mutation was found) results. After results disclosure, those with uninformative results reported a significantly lower perceived risk of HBOC compared with baseline. There were no significant changes at 2-week follow-up in global or cancer-specific distress scores among those with either positive or uninformative results. Women who received positive test results reported an increased commitment to screening, and those who received uninformative results maintained a similar commitment to screening compared with baseline. Following genetic counseling for results disclosure, participants correctly answered a significantly greater number of HBOC knowledge items (93%) compared with baseline (71%). Our findings indicate that disclosure of either positive or uninformative genetic test results does not increase psychological distress. Genetic counseling improved participants' knowledge of HBOC and may facilitate understanding of the importance of screening adherence. Although women who received uninformative results may inappropriately perceive their HBOC risk to be lowered, intention to continue screening did not decline. Our data show that additional counseling and education may be required to facilitate an accurate understanding of the meaning of uninformative test results, and that follow up also may be needed to determine whether perceptions of personal HBOC risk are modified over time.

Program Nr: 1776 from the 1999 ASHG Annual Meeting

The cell line W12 as a model for investigation of genomic events associated with the progression of cervical squamous cell carcinoma. *M.R. Pett[1], I.R. Roberts[1], M.A. Stanley[1], N. Coleman[1,2].* 1) Department of Pathology, University of Cambridge, Cambridge, United Kingdom; 2) Department of Histopathology, Addenbrooke's Hospital, Cambridge, United Kingdom.

Although the association of human papillomavirus (HPV) infection with cervical squamous cell carcinoma (SCC) is well recognised, the nature of any additional genomic abnormalities that occur during neoplastic progression remains uncertain. The cell line W12 has been generated from a low-grade squamous intra-epithelial lesion of the cervix (CIN1) and contains the HPV-16 genome in episomal form. At early passage W12 recapitulates a CIN1 phenotype when grafted onto nude mice but following long-term passage there is viral integration and development of a high-grade (CIN3) phenotype. W12 therefore represents a suitable cell line for the study of genomic events associated with progression of cervical SCC. We have used spectral karyotyping and comparative genomic hybridisation to identify genomic events that occur during long term passage of W12. Loss of 10p and gain of 10q and 20p are early events that are stably maintained. Loss of 3p, gain of 3q and amplification of 5p are later events that occur following long term passage and which correlate well with observations made using clinical material. We are currently investigating whether the development of thaese genomic changes correlates with specific alterations in the phenotype of W12.

Program Nr: 1777 from the 1999 ASHG Annual Meeting

10q LOH analysis and PTEN mutations in melanoma cell lines and tumours. *P.M. Pollock, G.J. Walker, N.C. Bloch, T. Que Noy, N.K. Hayward.* Cancer Unit, QIMR, Brisbane, Queensland, Australia.

Cytogenetic and loss of heterozygosity (LOH) studies have indicated that one or more tumour suppressor genes (TSGs) mapping to chromosome 10q may be involved in the development of sporadic melanoma. A recently identified TSG, PTEN, which maps to chromosome band 10q23 has been shown to be mutated/deleted in a wide range of tumours and cell lines including melanoma. To further characterise the role of PTEN in melanoma we screened 57 melanoma cell lines for homozygous deletions and mutations by a combination of genomic PCR, RT-PCR and sequencing. We also performed microsatellite analyses to detect regions of 10q hemizygosity in these cell lines. Five melanoma cell lines were found to carry exon specific homozygous deletions. One cell line demonstrated a larger fragment by RT-PCR, corresponding to a 52bp insertion between exons 2 and 3 causing premature truncation of PTEN. Direct sequencing revealed 8 missense mutations in seven cell lines. When the results of this study are combined with previous findings,overall, homozygous deletions and mutations in the PTEN gene have been detected in 27/144 (19%) and 18/144 (13%) melanoma cell lines respectively. To further assess the involvement of PTEN in melanoma tumours, detailed LOH analysis was performed using 14 microsatellite markers mapping to chromosome 10, and a panel of 33 melanoma tumours. 8/33 tumours demonstrated loss at all informative markers, while another 4 tumours had partial loss restricted to 10q. Exons 1-9 of PTEN were sequenced in all tumours demonstrating loss surrounding this locus. An 11bp deletion within exon 7 was detected in one tumour. In summary, while approximately half of the melanoma cell lines were hemizygous for 10q, only 23% were found to carry a mutation or deletion in the remaining PTEN allele and only 1/10 tumours showing LOH carried a mutation. This low frequency of mutations in both cell lines and tumours demonstrating hemizygous loss, combined with the presence of a minimal region of loss telomeric to PTEN in melanoma cell lines, supports the notion that an additional locus telomeric to PTEN is involved in melanoma progression.*PTEN*.

Program Nr: 1778 from the 1999 ASHG Annual Meeting

Comprehensive molecular analyses of the APC gene of FAP patients; Novel mutations and relation to phenotype.

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Colorectal cancer is one of the most common causes of cancer-related deaths worldwide. A small percentage of all colorectal cancers are due to Familial Adenomatous Polyposis Coli (FAP) which is an autosomal dominantly inherited syndrome. Mutations in and/or loss of expression of the APC gene on chromosome 5q21 predispose at-risk individuals by initiating polyp formation and progression to carcinogenesis. Genotype/phenotype correlations with FAP have been reported for extracolonic disease, most notably CHRPE and desmoid growth. As the vast majority of APC mutations reported to date are either nonsense or frameshift mutations which result in a truncated protein product, the Protein Truncation Test (PTT) is often employed in screening programs for FAP patients and at-risk family members.

A comprehensive study of the APC gene of FAP patients, was undertaken using PTT as the initial screening tool and Restriction Endonuclease Fingerprinting or Single Strand Conformation Analysis as secondary methods, followed by automated sequencing. Sixteen of the 25 mutations identified so far from individuals have been novel. Of these, a deletion mutation (5819-5823delTACCA, frame shift A1946X) is particularly interesting in that it was identified in a family with Hereditary Desmoid Disease and is outside of the region (codons1403-1924) of the APC gene commonly associated with extracolonic desmoid growth.

Our data suggest that complete mutation characterization including sequence verification within FAP patients offers more information for genetic counseling, in some cases offers some prediction of clinical outcome, and often encourages presymptomatic testing of at-risk family members.

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Program Nr: 1779 from the 1999 ASHG Annual Meeting

Cloning and characterization of genes from Wilms' tumor candidate regions. *D. Prawitt[1], C. Spangenberg[1], G. Klemm[1], J. Truebenbach[1], B. Gaertner[1], T. Enklaar[1], M. Higgins[3], J. Pelletier[2], A. Winterpacht[1], B.U. Zabel[1].* 1) Molec Genetics Lab, Children's Hosp/ Univ Mainz, Mainz, Germany; 2) McGill Cancer Center, McGill University of Montreal, Canada; 3) Roswell Park Cancer Center, Buffalo, NY, USA.

Wilms'tumor (WT) or nephroblastoma is the predominant solid childhood tumor with an incidence of 1:10000. The genetic heterogeneity of this renal neoplasia is well established but up to now no more than one responsible gene (WT1 of chromosomal region 11p13) could be identified. Only 10-15% of WTs display functional WT1 mutations and the search for additional genes involved in tumorigenesis is still in progress. Several genomic candidate regions were identified by the detection of WT specific chromosomal abnormalities, such as loss of parts of chromosome 11p, 16q, 17q or gain of chromosome 1, 6, 7p and 12q (Mitelman et al., 1997, Cancer Res) as well as WT specific translocation breakpoints (Mitelman et al., Nature Genet 1998).

To detect and characterize genes with potential involvement in WT development and progression we used a positional cloning strategy and differential display analysis comparing adult and fetal kidney as well as WT tissues.

The genomic approach was based on tumor cell lines with specific translocations: RH16 [with t(11;22)(p15.5;q11.23)] and WT128 [with t(7;12)(p22;q22)]. We isolated breakpoint-spanning genomic clones which are tested for disrupted genes to identify candidate genes. Differential display experiments led to ESTs serving as starting points for further analysis. We used a chromosomal hybrid panel and FISH analysis in order to concentrate on clones mapping to regions associated with WT. Up to now we were able to identify three ESTs mapping to chromosome 1, one of them being a clone for the TM7SF1 gene. TM7SF1 codes for a putative seven pass transmembrane receptor, downregulated in the majority of tumors. The two other clones (10C2 and 6A2) were either highly expressed in normal kidney (10C2) or upregulated in WT (6A2). We are currently obtaining full length cDNA clones for the candidate ESTs.

Program Nr: 1780 from the 1999 ASHG Annual Meeting

Human *PTTG*: a new proto-oncogene family. *T.R. Prezant[1,2], P. Merkel[1], P. Kadioglu[1], A.P. Heaney[1], G. Horwitz[1], S. Melmed[1,2]*. 1) Dept Med, Div Endocrinology, Cedars-Sinai Medical Ctr, Los Angeles, CA; 2) UCLA School of Medicine, Los Angeles, CA.

We recently isolated human *PTTG* (Pituitary Tumor Transforming Gene). Overexpression of the 609 bp coding sequence transforms cells *in vitro*, causes tumors in athymic mice and induces the transcription of basic FGF, an angiogenic protein. These three functions depend on an intact P-X-X-P motif that can potentially bind to SH3 domains. Human *PTTG* expression is increased in pituitary tumors and in many types of cancer cell lines, and is highly expressed in normal testis. While localizing human *PTTG* to chr. 5q33 by radiation hybrid (RH) mapping, we discovered a second intronless *PTTG* gene on chr. 4p12. This location was confirmed by PCR and Southern blot analysis of DNA from a human/hamster hybrid cell line containing human chromosome 4. The existence of *PTTG2* suggested that the two genes might have differential expression in normal development and tumorigenesis. We tested this by PCR-ELISA and dideoxy fingerprint analyses of *PTTG* cDNAs from normal tissues, pituitary tumors, and cancer cell lines. *PTTG1* (the intron-containing gene) is the major species in normal cells, primary tumors and cancer cell lines. However, *PTTG2* is expressed in several normal tissues and has increased transcription in some pituitary tumors. Through sequence analysis of genomic DNA for the *PTTG2* studies, we found 37 homozygous sequence changes in a control. Primers specific to this new sequence were used to repeat RH mapping analysis, with the discovery of a third member of the *PTTG* family, which maps to chromosome 8 and, like *PTTG2*, is intronless in the coding region. While testing "common primers" designed for quantitative expression studies, we amplified three additional *PTTG*-related sequences from genomic DNA. *PTTG4L* is intronless, while the other two products are larger. Primer extension analysis has shown that *PTTG2* and *PTTG3* are expressed in normal colon, but that the large increase in *PTTG* expression in adjacent colon tumors is due to *PTTG1*. Thus, this family of proto-oncogenes appears to differentially participate in tumor-specific pathogenesis.

Program Nr: 1781 from the 1999 ASHG Annual Meeting

OVCA1, a novel tumor suppressor, is aberrantly expressed in ovarian carcinomas. *A.H. Prowse, W. Bruening, A.K Godwin.* Fox Chase Cancer Center, Philadelphia, PA.

We have identified a gene, *OVCA1*, within a region of minimal allelic loss in ovarian tumors at 17p13.3. *OVCA1*codes for a highly conserved protein with no known function. Previous northern and western blot studies have revealed that *OVCA1*mRNA and protein levels were down-regulated in ovarian tumors as compared to normal human ovarian surface epithelial cells. In addition, overexpressing OVCA1 in the ovarian cancer cell A2780 resulted in dramatically reduced rates of proliferation which correlated with an increased proportion of the cells in the G1 fraction of the cell cycle and decreased levels of cyclin D. Localization studies indicate that OVCA1 shuttles between the cytoplasm and nucleus in a cell cycle dependent manner. We have now performed immunohistochemistry on normal ovaries and benign and malignant ovarian tumors using antibodies derived to the N and C-termini of OVCA1. In normal ovaries and ovarian tumors of low malignant potential, strong nuclear and cytoplasmic staining in the epithelial cells was observed using both antibodies. In contrast, 90% (9/10) ovarian adenocarcinomas showed little or no cytoplasmic staining using the N-terminal antibody. However, varying intensities of nuclear staining (high/medium in 5/9 tumors and light staining in 4/9 tumors) was observed. Interestingly, no nuclear or cytoplasmic staining was observed with the C-terminal OVCA1 antibody in these sections. Furthermore, there was no apparent correlation with cellular proliferation (as determined by ki-67 expression) and OVCA1 expression in these carcinomas. Our results suggests that in ovarian adenocarcinomas, OVCA1 undergoes an aberrant post-translational modification (reflected by a loss in immunoreactivity of the C-terminal antibody) which results in the mislocalization of OVCA1 to the nucleas and exclusion from the cytoplasm.Studies are underway to determine the molecular mechanisms regulating OVCA1 expression and localization in both normal and tumor tissue. These data suggest that *OVCA1*may represent a novel class of tumor suppressor genes and altered expression and/or post-translational modification of OVCA1 contributes to the development of ovarian tumors.

Program Nr: 1782 from the 1999 ASHG Annual Meeting

Polymorphic Variation at the BAT-25 and BAT-26 Loci in Individuals of African Origin: Implications for Microsatellite Instability Testing. *R.E. Pyatt[1], R.B. Chadwick[2], C.K. Johnson[2], C. Adebamowo[3], A. de la Chapelle[2], T.W. Prior[1]*. 1) Pathology, Ohio State University, Columbus, OH; 2) Division of Human Cancer Genetics, Ohio State University, Columbus, OH; 3) Dept. of Surgery, University College Hospital , Ibadan, Nigeria.

Instability in the repeat size of microsatellite sequences has been described in both hereditary nonpolyposis and sporadic coloretcal cancers. Tumors expressing microsatellite instability are identified through the comparison of the repeat sizes at multiple microsatellite loci between tumor and matched normal tissue DNA. The use of a five marker panel including two mononucleotide repeat microsatellites, BAT-25 and BAT-26, has recently been suggested for the clinical determination of tumor microsatellite instability. The BAT-25 and BAT-26 loci included in this panel have both demonstrated sensitivity to microsatellite instability and normal quasi-monomorphic allelic patterns which has simplified the distinction between normal and unstable alleles. However in this study, we identified allelic variations in the size of the poly (A) tract at BAT-26 in 12.6% of 103 healthy African-Americans screened. In addition, 18.4% exhibited allelic size variations in the poly (T) tract at BAT-25. Finally, 2.9% showed variant alleles at both BAT-25 and BAT-26 loci. Screening a population of Nigerians confirmed the polymorphic nature of both loci and the ethnic origin of alleles not identified in other populations studied thus far. Our results dispute the quasi-monomorphic nature of both BAT-25 and BAT-26 in all populations and support the need for thorough population studies in order to define the different allelic profiles and frequencies at microsatellite loci. Additionally, we recommend that matched normal tissue DNA be required for the comparison of test results for all samples showing microsatellite sequences suggestive of microsatellite instability.

Program Nr: 1783 from the 1999 ASHG Annual Meeting

Exploring The Dysregulation of *HMGIC* In Uterine Leiomyomata with t(12;14). *B.J. Quade[1], M. Getman[2], T.-Y. Wang[1], S. Weremowicz[1], P. Dal Cin[1], C.C. Morton[1,2].* 1) Depts. of Pathology and; 2) OB/GYN, Brigham & Women's Hospital and Harvard Medical School, Boston, MA.

Uterine leiomyomata (UL) are one of several benign mesenchymal tumors characterized by frequent chromosomal rearrangements involving the DNA architectural factor *HMGIC* in 12q15. In UL, 12q15 is translocated with 14q23-24. It has recently been suggested that t(12;14) produces fusion gene products containing *HMGIC* and *RAD51B*, a recombination repair gene family member. We previously mapped the chromosome 12 breakpoints centromeric (5') to *HMGIC* in 83% of tumors and have shown that t(12;14) dysregulates *HMGIC*, resulting in expression of its full coding sequence. To explore the possibility that t(12;14) involves *RAD51B*, we designed a series of primer pairs to hypothetical fusion products containing *HMGIC* and *RAD51B* in both orientations, and analyzed a series of nine t(12;14) UL by RT-PCR. In almost all cases, RT-PCR with *RAD51B*-specific primer pairs produced the expected product, consistent with either the normal *RAD51B* transcript or a *RAD51B-HMGIC* fusion transcript 5' to the fusion. No transcripts containing the 5' portion of *RAD51B* fused to the 3' of *HMGIC* were detected. In only one case was a fusion product containing *HMGIC* exons 1-3 and *RAD51B* exons 8-11 detected. This tumor was unique because it was one of a minority (17%) with rearrangement in the 3' region of *HMGIC* by FISH analysis. mRNA from this tumor was also analyzed by 3' RACE using a *HMGIC*-specific 5' primer. A novel *HMGIC* fusion transcript was detected in which an ectopic 244 bp were fused to the 3' UTR of *HMGIC* at nt 1912. The 244 bp sequence was mapped to chromosome 14 using radiation hybrids. To understand better the two different fusion transcripts found in this tumor, we isolated the breakpoints from the der(12) and der(14) chromosomes and found that they corresponded to the junction in the fusion transcript with the 244 bp sequence. The predominance of breakpoints 5' to *HMGIC* in UL suggests that the primary mechanism may be "promoter swapping." In our series of UL, only rare breakpoints involving the 3' region of *HMGIC* may produce fusions with either anonymous sequences at the breakpoint or *RAD51B* and *HMGIC*.

Program Nr: 1784 from the 1999 ASHG Annual Meeting

HER-2/neu Amplification in Simple & Complex Endometrial Hyperplasias.. *M.R. Quddus, A.J. Tsai, C.J. Sung, M.M. Steinhoff, U. Tantravahi.* Departments of Pathology & Molecular Cytogenetics, Women & Infants' Hospital, Providence, RI.

Her-2/neu amplification has been shown to be predictive of poor prognosis in various gynecologic cancers including breast & endometrial cancers. Hyperplasia of the endometrium can progress from simple to complex histologic forms and eventually, to frank carcinoma. The identification of genetic changes in these precancerous states may help elucidate the pathogenesis of the disease. The current study evaluated Her-2/neu amplification as a possible early event in the loss of control of cell proliferation in endometrial hyperplasia.

Fifty-six archival paraffin embedded endometrial hyperplasia tissues, 20 simple (SH), 21 complex (CH) & 14 complex hyperpalsia with atypia (CHA), were obtained from the Department of Pathology, Women & Infants' Hospital. Fluorescent *In-situ* Hybridization (FISH) was performed on 4m tissue sections. A DNA probe (Oncor) assessing gene amplification rather than gene product was used. Hybridization signals were detected using Digoxigenin Rhodamine Detection Kit. A total of 50 cells were scored in a blinded fashion using a fluorescent microscope with triple band filter. Cells with >4 signals were considered to have Her-2/neu gene amplification. Small amplification was defined with >4 distinct signals & large with >>4 signals that could not be counted individually. Adjacent normal tissue area signals were counted as an internal control.

The results indicated that 85% SH, 87.7% CH & 92.9% CHA cases showed Her-2/neu amplification. Thirty percent SH, 47.6% CH & 57% CHA showed large amplification.

Our study revealed Her-2/neu amplification may be an important factor in endometrial cancer progression. The amplification may be one of the early events in transformation of normal to hyperplastic state.

Program Nr: 1785 from the 1999 ASHG Annual Meeting

Absence of evidence for a familial breast cancer susceptibility gene at chromosome 8p12-p22. *N. Rahman, D. Teare, J. Chang-Claude, D. Easton, D. Eccles, D. Goldgar, J. Mangion, H. Meijers, S. Seal, Y. Shugart, M. Stratton, D. Thompson, B. Weber.* The BRCA3 linkage consortium, Section of cancer genetics, Institute cancer research, Sutton, Surrey, England.

Several recent studies indicate that the majority of families with five or fewer cases of breast cancer and no case of ovarian cancer are not due to BRCA1 or BRCA2. It has been proposed that a further breast cancer susceptibility gene that may account for some of these families is located on chromosome 8p12-p22. We have identified 38 site-specific breast cancer families that have greater than 80% posterior probability of being due to genes other than BRCA1 and BRCA2. These families have been examined for linkage to 8p12-p22 using markers flanking the putative location of the gene. The overall multipoint LOD score is strongly negative across the whole 44cM. At marker D8S136 the LOD score is -9.43. The individual multipoint score is negative in 25 of the 38 families and only exceeds 0.5 in a single family, (with a multipoint lod of 1.22). The maximum heterogeneity lod score was 0.03 at marker D8S136 with estimated proportion linked (a) of 3% (95% confidence interval 0%-28%). These data do not lend support to the hypothesis that chromosome 8p12-p22 harbors a familial breast cancer susceptibility gene.

Program Nr: 1786 from the 1999 ASHG Annual Meeting

A FISH study of the variant Philadelphia rearrangements. *K.S. Reddy, V. Sulcova.* Dept Cytogenetics, Quest Diagnostics, Inc, San Juan Capist, CA.

A total of 39 variant Philadelphia (Ph) translocations were studied by fluorescence in situ hybridization (FISH) using MBCR/ABL, mBCR/ABL or DBCR/ABL probes. Seven cases did not have a BCR/ABL fusion signal. Out of a total of 32 fusion positive cases, 5 were simple variants involving chromosome 22 and another chromosome; 23 were complex variants involving chromosomes 22, 9 and a third chromosome (18 cases) or 22, 9, and two other chromosomes (4 cases). Masked Philadelphia rearrangements were detected in 4 cases. One case was a Philadelphia chromosome mimic. FISH has become a widely used method for studying Philadelphia rearrangements. The expected pattern for DBCR/ABL probe is one green ABL signal [1G] on the normal 9, one red BCR signal [1R] on the normal 22, and two fusion signals BCR/ABL and ABL/BCR [2F] on a derivative 22 and a derivative 9, respectively. Deviant patterns from 1G1R2F and sometimes 1G1R2F are indicative of a variant, as long as there is a fusion signal. However, in interphase analysis it is not possible to visualize a variant rearrangement and when a deviant pattern involving at least one fusion signal is observed the following possibilities should be contemplated. The different patterns observed in fifteen Ph variants studied with DBCR/ABL probe were 2G2R1F (40%), 1G1R2F (20%), 1G1R1F (20%), 1G2R1F (13.3%) and 2G1R1F (6.66%). A 2G2R1F, FISH pattern observed in 6 cases appears to involve a single concerted event of simultaneous breaks on the participating chromosomes followed by mismatched joining. Three cases with 1G1R2F most probably arose by two independent sequential events. The pattern of 1G1R1F suggests either the BCR and ABL breakpoints are different or there are deletions at the breakpoints since residual signals are not observed. Two independent events appear to be involved in 1G2R1F with a reverse cryptic 9,22 rearrangement as the first event. In one case 2G1R1F was observed and the plausible explanation for this pattern in our case is an insertion of ABL next to BCR and either a simultaneous or a sequential translocation with another chromosome.

Program Nr: 1787 from the 1999 ASHG Annual Meeting

Analysis of the human mismatch repair genes, hMSH2 & hMLH1 in an early onset colon cancer population. *C. Reeves[1], M. Hepburn[1], A. Schmitt[1], J.P. Terdiman[2], G.A. Miller[1]*. 1) Molecular Profiling Laboratory, Genzyme, Framingham, MA; 2) University of California San Francisco, San Francisco, CA.

Hereditary Non-Polyposis Colon Cancer(HNPCC) is characterized by the early onset of colorectal cancer(CRC), proximal colonic tumors, an increased frequency of multiple primaries and an autosomal dominant genetic transmission. The frequency of the disease ranges between 3 and 6% of all colorectal malignancies. HNPCC is thought to be related to abnormalities in any one of at least five genes that normally assist in repairing DNA damage. Of the mismatch repair(MMR) genes, mutations in hMSH2 and hMLH1 account for the majority of the reported cases.

Using Denaturing Gradient Gel Electrophoresis and Automated Fluorescence Sequencing our laboratory has developed a high through-put, cost effective means to screen for mutations within the MMR genes, hMSH2 and hMLH1. Individuals were selected for testing on the basis of family history, microsatellite instability status and the total number of known risk factors for HNPCC. These risk factors include: greater than 2 CRC in a family; earliest age of diagnosis being less than 40; endometrial cancer in the family and other HNPCC related cancers in the family. To date we have studied 33 families and found 17 unique mutations. 13 of 17 mutations resulted in either a stop codon or the alteration of a splice site. The remaining mutations were amino acid changes of unknown significance. These results agree with previous studies indicating the presence of mutations in the MMR genes in approximately 50% of the HNPCC cases.

Our results demonstrate that the careful selection of individuals for testing and the use of highly sensitive and accurate methods of detection can provide valuable information to individuals at risk for HNPCC.

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Program Nr: 1788 from the 1999 ASHG Annual Meeting

Analysis of E-cadherin (*CDH1*) and transforming growth factor-b **type II receptor (*TGFBR2*) genes in genetic predisposition to early onset colorectal carcinoma.** *F.M. Richards[1], L. Verma[1], T.R. Porter[1], D.G.R. Evans[3], E.R. Maher[1,2]*. 1) Medical & Molecular Genetics Section, Division of Reproductive & Child Health,University of Birmingham, The Medical School, Edgbaston, Birmingham, B15 2TT, UK; 2) West Midlands Regional Clinical Genetics Service, Birmingham Women's Hospital, Edgbaston, Birmingham, B15 2TG, UK; 3) Department of Medical Genetics, St Mary's Hospital, Manchester, M13 0JH, UK.

Colorectal cancer susceptibility is genetically heterogeneous. In patients with HNPCC most Amsterdam positive cases have germline mismatch repair (MMR) gene mutations, usually in *MSH2* or *MLH1*. In addition, germline *MSH2* and *MLH1* mutations account for a small proportion of patients with non-familial early onset colorectal cancer (EOCC). However the absence of germline MMR mutations in some HNPCC families and most EOCC and non-Amsterdam criteria familial colorectal cancer kindreds is consistent with additional susceptibility genes. Recently we have suggested that germline E-cadherin (*CDH1*) gene mutations predispose to both early onset colorectal cancer and familial gastric cancer (Hum. Mol. Genet. 8: 607-610; 1999). In addition, germline transforming growth factor-b type II receptor (*TGFBR2*) gene mutations have been reported in atypical HNPCC kindreds. To investigate the role of germline *CDH1* and *TGFBR2* mutations in colorectal cancer susceptibility we analysed these two genes in (a) 23 cases of microsatellite stable (MSS) EOCC (<50 years) and (b) 21 HNPCC families without detectable *MSH2* or *MLH1* mutations. No germline *CDH1* mutations were identified in either group. Similarly no *TGFBR2* mutations were detected. These results suggest that germline *CDH1* and *TGFBR2* mutations are not a major cause of HNPCC or early onset MSS colorectal cancer.

Program Nr: 1789 from the 1999 ASHG Annual Meeting

Exon Deletions and Duplications in BRCA1 Detected by semi-quantitative Polymerase Chain Reaction. *M.D. Robinson[1], C.E. Chu[2], G. Turner[2], D.T. Bishop[3], G.R. Taylor[1]*. 1) Yorkshire DNA Laboratory, St James's Hosp, Leeds, England; 2) Clinical Genetics Service, St James's Hosp, Leeds; 3) ICRF Genetic Epidemiology Laboratory, St James's Hosp, Leeds.

The majority of mutations identified in the BRCA1 gene lead to the production of a truncated product . Most studies have performed mutation analysis on genomic DNA using PCR based techniques such as heteroduplex analysis and the protein truncation test (PTT). Several recent reports have described partial deletions and in one case a partial duplication of the BRCA1 gene. These rearrangements would have been missed by conventional PCR based methods and were detected either by reverse transcriptase PCR (RT-PCR) or Southern blotting. RT-PCR analysis has been shown to give variable amplification of mutant alleles, which could lead to rearrangements being missed if testing is by this method alone. A recent study concluded that Southern blot analysis was not sensitive enough to detect partial gene duplications unless they produced an abnormal restriction fragment pattern. We have developed a semi-quantitative polymerase chain based fluorescent assay for the detection of previously identified deletions. This assay was used to screen 44 cases from high-risk families within the Yorkshire Health Region. No deletions were detected but five cases (11%) with an apparent duplication of exon 13 in BRCA1 were identified.

Program Nr: 1790 from the 1999 ASHG Annual Meeting

Molecular genetics of recurrent pituitary neoplasia. *J. Rock[1], S. Mehra[2], K. Ho[2], W. Chandler[3], P. Mckeever[3], K. Pindolia[2], M.J. Worsham[2].* 1) Neurosurgery, Henry Ford Health Systems, Detroit, MI; 2) Cancer Genetics Research, Pathology, Henry Ford health Systems, Detroit, MI; 3) University of Michigan, Ann Arbor, MI.

Pituitary tumors are, in general, benign, slow growing neoplasms. Regrowth after treatment is not uncommon and predicting the biologic potential of a given tumor based on histologic appearance is unreliable. While the frequency of malignant transformation is very low in individual neoplasms, the lesions tend to recur locally. To better understand the underlying biological behavior of tumor progression/recurrence we studied a patient with a primary adenoma and three subsequent recurrences. In addition to cytogenetics, to explore at a subcellular level the molecular characteristics of the primary and the recurrent tumors, we evaluated the Her2/neu oncogene for gene amplification, status of p53 expression, and the 11q13 region for loss of heterozygosity (LOH) with markers in the vicinity of the MEN1 gene. Though many studies have demonstrated a prognostic role for Her2/neu gene amplification in breast, ovarian and colon cancers, its role in pituitary tumorigenesis is largely unknown. The patient was diagnosed with Cushing's Disease at age 33 and radiated. Subsequent progression led to three surgical resections over 12 years. Cytogenetic studies revealed an abnormal hypodiploid karyotype with several chromosomal rearrangements and losses, including both copies of chromosome 11. Fluorescent in situ hybridization with probes to the centromere of chromosome 17 and Her2/neu suggested a trend toward slightly increased copy number for the Her2/neu gene with an average ratio of 1.4 in the primary and recurrent adenomas as compared to the normal ration of 0.92. LOH results of four markers in the 11q13-11q23.2 region suggested instability of this region. Two of the four markers in the second and third recurrences indicated an allele pattern that was different from the normal pituitary cells and that of the primary adenoma. The latter suggests that instability of this region is important in the progression of pituitary adenomas.

Program Nr: 1791 from the 1999 ASHG Annual Meeting

Characterization of a novel member of the Dbl family of Oncogenes. *N.R. Rodrigues[1], A.M. Theodosiou[2], M.A. Nesbit[3], A.T. Tandle[4], D. Saranath[4], K.E. Davies[1]*. 1) Dept of Human Anatomy & Genetics, Univ Oxford, Oxford, England; 2) Section of Gene Function and Regulation, Institute of Cancer Research, London, England; 3) MRC Molecular Endocrinology Group, MRC Clinical Sciences Centre, Hammersmith Hospital, London, England; 4) Laboratory of Cancer Genes, Cancer Research Institute, Bombay India.

We have identified a novel member of the family of Dbl oncogenes, *Ngef* . Many members of this family have been shown to function as guanine nucleotide exchange factors (GEF) for the Rho-type GTPases. Unlike the other members which show a wider tissue distribution, including hematopoietic tissues, this gene shows predominant expression in brain, with the strongest signal in the caudate nucleus. This region is associated with the control of movement and undergoes specific degeneration in disorders such as Huntington's disease. *Ngef* contains a translated trinucleotide repeat, a polyglutamine stretch interrupted by a glycine. We have isolated both the human and mouse homologues of *Ngef*. We have localized *Ngef* in mouse and human. We have shown that *Ngef* has transforming activity in cell culture and is able to induce tumors in nude mice.

Program Nr: 1792 from the 1999 ASHG Annual Meeting

Complex rearrangement in *BRCA1* results in the in-frame loss of exons in the BRCT domain. *E.M. Rohlfs[1], Q. Yang[2], C. Skrzynia[2,3], M.L. Graham[2,3], L.M. Silverman[1]*. 1) Dept Pathology & Lab Medicine; 2) Lineberger Comp Cancer Center; 3) Dept Medicine, Univ of North Carolina, Chapel Hill, NC.

In a linkage study of *BRCA1* families it was estimated that as few as 63% of the *BRCA1* mutations were identified by techniques which study individual exons and intron/exon borders. Genomic rearrangements and promoter mutations may account for the remainder; one study has shown that 15% of the mutations in their breast/ovarian cancer families were genomic rearrangements. Most frequently, the rearrangements result from homologous recombination between *Alu* repeats resulting in deletion, duplication or inversion of genomic sequence. Protein truncation analysis of lymphocyte RNA from a woman with breast cancer (dx age 51) and a family history of breast and ovarian cancer in her mother (37, 41) and aunt (70, 83), identified a truncated protein within the segment which corresponds to exons 12 - 24. This resulted from a shortened RT-PCR product which sequencing showed lacked exons 17 - 20. Loss of these exons results in the deletion of 97 amino acids but maintains the reading frame. Sequence analysis of all exons did not identify a mutation in any splice junctions. By southern blot analysis it was determined that ~11.5kb of genomic DNA between exons 16 and 21 was deleted. Amplification of genomic DNA identified a junction fragment that also indicated an 11.5kb deletion. However, sequence analysis of this fragment identified an 8216 bp deletion with breakpoints within AluSp (Int 16) and AluSx (Int 19) repeats. An additional junction fragment between introns 19 and 20 has been isolated and appears to account for the remaining ~3.3kb deletion. These tandem deletions result in loss of exons 17 through 20 at the RNA level. The region of the BRCA1 protein that is encoded by these exons (amino acids 1663 to 1759) corresponds to a subregion of the BRCT domain that interacts with RNA helicase A and thereby RNA polymerase II (amino acids 1650 to 1800). The binding of these proteins are thought to be the basis of *BRCA1*'s transcriptional activation function. This is the first report of an in-frame deletion in a high-risk breast cancer individual that removes a putative functional domain of BRCA1.

Program Nr: 1793 from the 1999 ASHG Annual Meeting

Genetic changes in hereditary prostate cancers by comparative genomic hybridization. *A. Rokman[1], J. Schleutker[1,2], P. Koivisto[1], M. Matikainen[1], R. Karhu[1], O. Kallioniemi[1,2].* 1) Lab. Cancer Genetics, IMT, TAUH, Univ. Tampere, Tampere, Finland; 2) Cancer Genetics Branch, NHGRI, NIH, Bethesda, MD.

According to the Knudson's two hit hypothesis, cancer predisposing germline alterations are uncovered by somatic chromosomal losses that inactivate the wild-type alleles of recessive tumor suppressor genes. The search for deletions in tumor tissues from patients with an inherited predisposition may therefore highlight putative cancer susceptibility loci (Hemminki et al., 1997). Here, we used comparative genomic hybridization (CGH) to analyze chromosomal alterations in 21 primary prostate cancers from men belonging to Finnish prostate cancer families. The average number (± S.D.) of chromosomal alterations per tumor was 4.0 ± 1.9 with 2.0 ± 2.2 losses and 2.0 ± 2.1 gains. The most common losses were found at chromosomal regions 13q21-q22 (38%), 8p12-pter (31%), 6q14-q21 (19 %), 11q22-q23 (10%), 19p (10 %) and 21q (10%), and the most common gains at 7q11 (38%), 19p (25%), 8q (10%), 9q (10%) and 1p33-pter (10%). The comparison of these data with our previous studies of unselected primary prostate cancers (Visakorpi et al., 1995) indicates that genetic changes do not appear to be significantly different between hereditary and sporadic prostate cancers. Analysis of another tumor material from patients belonging to large families linked to the HPC1 and HPCX loci is in progress.

Program Nr: 1794 from the 1999 ASHG Annual Meeting

Near-triploidy in chronic myelogenous leukemia. *B. Roland.* University of Calgary, Calgary, AB, Canada.

This report describes a case of chronic myelogenous leukemia (CML) with a near-triploid karyotype, and reviews the literature on similar cases. A 58-year-old female was diagnosed with CML in chronic phase. The bone marrow karyotype at diagnosis was 46,XX,t(9;22)(q34;q11.2)[18]/46,XX[2]. Three years later, when the patient was in accelerated phase, progressing to blast crisis, the karyotype was 65~69,XX,-X,-3,-4,-5,+6,-7,+8,-9,t(9;22)(q34;q11.2),+11,-12,-17,+18,+19,+20,+21,der(22)t(9;22)x2,+der(22)t(9;22)[cp19]/46,XX,t(9;22)[16]. The patient died one month later.

Although hyperdiploidy is common in CML at the time of progression, the presence of a near-triploid karyotype is rare, with only 7 cases reported previously. Review of the clinical features of the 8 patients shows that all had the near-triploid karyotype at the time of accelerated phase or blast crisis. There does not appear to be any association with gender (5 female, 3 male), age (41-68 years), length of chronic phase (3-9 years), or morphology at transformation. Review of the cytogenetic features of the 8 cases shows chromosome counts ranging from 58 to 71, with 2-4 copies of each chromosome, and 2-4 copies of the derivative chromosome 22. The chromosomes that most frequently show relative gains are chromosomes 8, 13 and 19 (7/8 cases each), and 6, 14 and 21 (6/8 cases each). The chromosomes that are most frequently under-represented are chromosomes 17 (8/8 cases), and 4, 5, 7, 9, and 12 (6/8 cases each). One or more structural abnormalities in addition to t(9;22) are also reported in 6 of the 8 cases.

Thus, the cytogenetic features of near-triploid CML are similar to the changes in hyperdiploid blast crisis, with the gain of chromosome 8, 19, 21, and der(22), and with the loss of 17p. However, near-triploidy differs in the higher frequency of structual abnormalities other than t(9;22), and in many of the relative chromosome gains and losses.

Program Nr: 1795 from the 1999 ASHG Annual Meeting

Using spectral karyotyping (SKY) for analysis of structural instability of chromosomes in human ovarian carcinoma cell lines. *A.V. Roschke[1], G. Tonon[1], K. Nakahara[1], D.A. Scudiero[2], I.R. Kirsch[1]*. 1) Gen Dept, Div Clinical Sci, National Cancer Inst, Bethesda, MD; 2) FCRDC, National Cancer Inst, Frederick, MD.

Structural chromosome rearrangements can be a characteristic of primary tumor samples, short-term cultures and established tumor cell lines. Most attention has been paid to non-random chromosomal aberrations whose recurrent presence is suggestive of a role in tumor progression or etiology. Nonrecurrent chromosomal aberrations usually are considered as noise that may reflect an underlying mechanism of instability, but otherwise are not significant. In this study we attempted to assess overall structural chromosomal instability (as manifested by the presence of recurrent and nonrecurrent chromosomal aberrations and the persistence of each over time) in six ovarian carcinoma cell lines (OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, SKOV-3 and IGROV-1) using spectral karyotyping, an advanced cytogenetic technique, which allows nonambiguous identification of each marker chromosome in each analyzed metaphase. Ovarian carcinoma cell lines used in our study had highly abnormal karyotypes with total numbers of marker chromosomes ranging from 15 to 56 per cell line. The origin of all marker chromosomes was defined and the frequency of their occurrence within distinct metaphases of the same cell line was estimated. As a measure of structural chromosomal instability we calculated for each ovarian cancer cell line a coefficient of structural instability equal to the proportion of rearranged chromosomes to the total number of chromosomes per cell, and a coefficient of marker instability equal to the proportion of variable versus consistent markers per cell. Our findings indicate that ovarian carcinoma cell lines had notable differences in both the frequency and ongoing rate of chromosomal instability.

Program Nr: 1796 from the 1999 ASHG Annual Meeting

Loss of heterozygosity analysis in the chromosome 6q region in adenoid cystic carcinoma. *S. Rutherford[1], R. Saadut[1], CR. Marshall[1], CA. Rumpel[1], HR. Frierson Jr[1], CA. Moskaluk[2].* 1) Department of Pathology, University of Virginia, Charlottesville, VA; 2) Departments of Pathology and Biochemistry and Molecular Genetics, University of Virginia, Charlottesville, VA.

Adenoid cystic is one of the most common of malignancies that arise in the salivary gland. It is present in the submandibular and minor salivary glands to a much greater extent than in any other location. A high rate of local recurrence, perineural spread and late distant metastasis characterizes the tumor. Adenoid cystic carcinoma produces both abundant basement membrane material and glycosaminoglycans and may be linked to novel genetic mechanisms of differentiation. Previous chromosome transfer experiments indicating chromosome 6 involvement to suppress tumorigenic or metastatic activity of melanoma cell lines, provide support for the hypothesis of one or more tumor suppressor genes residing within the chromosome 6q region. Moreover, cytogenetic studies have identified chromosome 6 as a target for genetic deletion in adenoid cystic carcinoma with breakpoints being mapped to the 6q21-q25 region. The present study extends these findings by assaying for loss of heterozygosity using polymorphic microsatellite markers in paired tumor and normal samples of individuals. Results from PCR amplification of 16 markers in 23 specimens has found a 43% overall incidence of 6q loss within the 6q16.3-6q22.33 region. Our preliminary data suggests that there may be two non-overlapping regions of consensus deletion bounded between D6S292 and D6S287 and between D6S287 and D6S251 respectively. An apparent "hotspot" of deletion also appears to be occurring at the D6S287, D6S407 and D6S262 contiguous markers. The results of additional samples and markers within this region are currently being determined and will be presented.

Program Nr: 1797 from the 1999 ASHG Annual Meeting

Functional characterization of OVCA1, a putative tumor suppressor. *A.M Salicioni, W. Bruening, L. Vanderveer, A.K. Godwin.* Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, PA.

OVCA1 is a candidate tumor suppressor gene that maps to a highly conserved region on chromosome 17p13.3. Its loss of heterozygosity is a frequent event in breast and ovarian carcinomas. OVCA1 is highly conserved and exists in at least two forms, a 50 and an 85 kDa protein. Somatic mutations in *OVCA1* have been detected, but are rare. Two germline missense mutations have been found in breast cancer-prone women who have tested negative for a *BRCA1* and a *BRCA2* mutation. Attempts to create breast and ovarian cell lines that stably over-express OVCA1 have generally been unsuccessful. The clones that do express exogenous OVCA1 do so at very low levels, and have dramatically reduced rates of proliferation. This reduction in proliferation rate correlates with an increased proportion of the cells in the G1 fraction of the cell cycle, and decreased levels of cyclin D, which may be caused by an accelerated rate of cyclin D degradation. To help determine the function(s) of OVCA1, we used a two-hybrid screening approach to identify OVCA1-associating proteins. The most redundant clone, *BOV-1* (Binding to OVCA1-1), accounted alone for 30% of the total cDNA isolated, and encoded for an ORF of 221 amino acids of an unknown protein. The expression pattern of *BOV-1* mRNA was evaluated by multiple tissue Northern blotting; three major mRNA species were detected (~5.5 kb, ~3.2kb, ~1 kb). All three species encode for BOV-1, are the result of alternative splicing and are expressed in all tissues to varying degrees: the 1-kb transcript was most abundant in testis, heart, placenta, spleen, thymus, and lymphocytes. Like OVCA1, BOV-1 appears to be highly conserved evolutionarily. *S. pombe* and *C. elegans* encode hypothetical proteins with 48.8 % and 60.6% identity and 64.5% and 75.4% similarity at the amino acid level to BOV-1, respectively. This extensive sequence similarity makes it highly likely that the biochemical function of the BOV-1 homolog has been conserved from yeast and nematodes to humans. Experiments are currently underway to determine the biological significance of this interaction in the context of normal and abnormal cell growth.

Program Nr: 1798 from the 1999 ASHG Annual Meeting

Linkage of a pedigree drawing program and database to a program for determining *BRCA* mutation carrier probability. *S.R. Sand[1], D.S. DeRam[2], D.J. MacDonald[1], K.R. Blazer[1], J.N. Weitzel[1]*. 1) Clinical Cancer Genetics, City of Hope Cancer Center, Duarte, CA; 2) GDS, Southbend, IN.

Genetic testing for *BRCA* gene mutations is generally reserved for families where mutation probability exceeds a practical threshold. Tables for estimating the probability that a family carries a mutation have been developed from high risk clinic genotyping data (Couch *et al*., 1997, Frank *et al*., 1998). Key factors are age for breast cancer (BC) and ovarian cancer (OC) occurence. These models do not account for limitations in family structure or non-penetrance. BRCAPRO is a computer program that uses a Bayesian calculation to estimate the probability that either a *BRCA1* or *BRCA2* mutation is present in a family based on first and second degree family history of BC or OC (Parmigiani *et al*, 1998). Key variables include the prevalence of mutations and age-specific penetrance estimates. Data entry was difficult and slow in the beta version. A user-friendly data input vehicle was recently developed (CAGENE, Euhus *et al*) with a pedigree interface. However, the CAGENE database is not flexible and data input is limited to first and second-degree relatives. Progeny (Genetic Data Systems [GDS], South Bend, IN), is a flexible pedigree application tool with a built-in relational database. Our primary data includes extended family history (>3 generations) and medical history germane to studies. An export module was developed to transfer relational pedigree data directly from Progeny to the BRCAPRO program, circumventing the need for data entry into both the pedigree drawing and risk calculation programs. The export module was tested by comparing the BRCAPRO output from 20 extended pedigrees in our Progeny database to the same data entered manually into BRCAPRO and into CAGENE. The output data were concordant for all three methods. Manual data entry took 10 times longer than either alternative. Although the time required for CAGENE and Progeny was similar, there is no capability for comprehensive data storage in the former. The Progeny export filter allows us to use our primary data to determine *BRCA* mutation probabilities within 5 minutes, making it a practical cancer risk assessment tool.

Program Nr: 1799 from the 1999 ASHG Annual Meeting

Characteristic genetic abnormalities in ependymal tumours of different malignancy grades. *D. Sanoudou[1], K. Ichimura[1], M.A. Ferguson-Smith[2], V.P. Collins[1], N. Coleman[1]*. 1) Dept Pathology, Univ Cambridge, Cambridge, England; 2) Dept Veterinary Medicine,Univ Cambridge,Cambridge, England.

Ependymomas are glial in origin and comprise approximately 6% of all CNS tumours. They are believed to develop from the ependymal cell lining of the ventricular system. Three major ependymoma entities have been described : Sub-ependymomas (WHO grade I), Ependymomas (WHO grade II) and Anaplastic ependymomas (WHO grade III). Numerous ependymoma cases have been studied genetically and cytogenetically. However, the results have been contradictory and the mechanisms of pathogenesis remain unclear. In the present study comparative genomic hybridisation (CGH) and allelic imbalance analysis have been used for the investigation of abnormalities in 41 ependymomas (10 sub-ependymomas, 23 ependymomas grade II and 8 anaplastic ependymomas). Paediatric and adult cases were included in the analysis. Only 4 sub-ependymomas had chromosomal aberrations. These included loss on 1p, 6p and 6q, loss of chromosome 8, 19 and X, and gain of chromosome 7. All grade II ependymomas had chromosomal abnormalities. The most frequent aberrations were loss of chromosome 22 (54% of cases), followed by gain of 9p arm (45%), loss on 6q arm (41%) and gain of chromosome 5 (32%). Other anomalies involved gain of 1q and 8q arms, and chromosomes 4, 5, 7, 9, 12, 15, 16, 18, 20 and X. Losses of 1p arm and chromosomes 2, 3, 10, 11, 13, 14, 16, 17, 19, 20 and 21 were also seen. The most common aberrations in grade III ependymomas were gain of 1q arm (63% of cases) and loss of chromosome 22. Other frequent abnormalities included gain of chromosome 8 and loss of chromosomes 11, 16, 17 and 19. Gain was also seen in 1p and 4q arms, and chromosomes 2, 5, 9, 10, 12, 14, 15, 20 and X. Losses were also seen on 9q arm, and chromosomes 3, 5, 6, 7, 8, 10, 13, 14, 15, 18 and 21. Certain differences in chromosomal aberrations could be seen between paediatric and adult patients, and between tumours arising at different anatomical locations.

Program Nr: 1800 from the 1999 ASHG Annual Meeting

CA repeat stimulated recombination: The role of mismatch repair in an *E. coli* model. *M.F. Santibanez, R. Gangeswaran, J.M. Hancock.* MRC CSC, Hammersmith Hospital, London, U.K.

Tumours arising in patients with an inherited defect of the mismatch repair (MMR) pathway are karyotypically simple compared to MMR proficient tumours in equivalent locations. This correlates with a lower frequency of LOH, a process often involving recombination. Chromosomal instability behaves in a dominant manner when MMR proficient and deficient cells are fused [Nature 396:643-646;1998]. This suggests that tumours in HNPCC patients have a lower LOH frequency not only because they have mutator phenotype (obviating the requirement for other mutational mechanisms to achieve, for example, inactivation of tumour suppressor genes), but also because they lack a pathway promoting recombination as a consequence of the MMR defect. The MMR system itself could be responsible for the recombinogenic effect. Although a variety of structures could mediate such an effect, microsatellites seem attractive candidates because the dramatic increase in their mutation rate in cells lacking functional MMR indicates that these sequences are important physiological targets for this repair system, and it has been shown that such repeats can promote recombination. We tested the influence of CA microsatellites on recombination using two compatible plasmids in different *E. coli* strains. Each plasmid carried a kanamycin resistance gene inactivated by a frame shift mutation. The introduction of a $(CA)_{97}$ repeat 3 of the resistance marker in one of the plasmids produced a consistent increase (2-6 fold) in the recombination rate in MMR proficient strains. MMR deficient strains had a higher (5-13 fold) recombination rate for the non array carrying constructs as compared with the parental strains. However, the introduction of the repeat failed to increase the recombination rate in these strains. Our observations are consistent with a mechanism in which stimulation of recombination by CA repeats results from the creation of slippage products during replication. This leads to unpaired regions between parental and daughter strands, which can constitute substrates for mismatch repair. The action of the MMR system then produces secondary lesions that are repaired by recombination.

Program Nr: 1801 from the 1999 ASHG Annual Meeting

The cellular distribution of BLM, the Bloom's syndrome protein. *M.M. Sanz[1], M. Proytcheva[2], N.A. Ellis[3], W.K. Holloman[1], J. German[1]*. 1) Departments of Microbiology and Pediatrics, Cornell University Medical College, New York, NY; 2) Department of Pathology, Albert Einstein College of Medicine, New York, NY; 3) Department of Human Genetics, Memorial Hospital for Cancer and Allied Diseases, New York, NY.

The gene mutated in Bloom's syndrome (BS) encodes a 1,417 aa protein, BLM, which has DNA helicase activity and so is assumed in some way to manipulate DNA. A major question is, What is/are its role(s) in DNA replication, DNA repair, transcription, and, or, recombination, in somatic and, or, germ-line cells? BLM is absent from cells of most persons with BS, which leads to the conclusion that its activity impinges directly or indirectly on the maintenance of genomic stability. In order to obtain leads as to BLM's exact function(s), its distribution has been examined in cells in culture, synchronized and not, using BLM antibodies and immunofluorescence microscopy. In interphase cells, BLM is detected only in the nucleus. In early post-telophase cells it is barely visible. It then increases in amount throughout G1, being both diffuse and also concentrating in multiple (sometimes 20 or more) minute, punctate, brightly fluorescing centers. During S, several (often 10 or more) larger punctate accumulations of BLM become brightly fluorescent, distinctive in appearance from the minute accumulations; these often co-localize with the PML nuclear protein, and in a more transitory way with RPA (the single-strand-binding replication protein A). In addition to the discrete foci, larger but less intensively fluorescing, irregularly shaped aggregates of BLM form in many nuclei. At metaphase, the condensed chromosomes appear to be BLM-free, the BLM then being distributed throughout the entire cell where it fluoresces intensely. Because early G1 cells have so little BLM (above), the conclusion seems inevitable that this is another protein that is eliminated from the cell during late mitosis. These observations will be extended to search for other proteins that co-localize with BLM, and they are to be complemented by immuno- and straightforward biochemical analyses of BLM.

Program Nr: 1802 from the 1999 ASHG Annual Meeting

BRCA1 and BRCA2 families in Finland: haplotype and phenotype analysis and predominance of founder mutations. *L. Sarantaus[1], P. Huusko[2], H. Eerola[1], V. Launonen[2], P. Vehmanen[1], K. Rapakko[2], E. Gillanders[3], K. Syrjäkoski[3], T. Kainu[3], P. Vahteristo[1], K. Pääkkönen[4], J. Hartikainen[5], C. Blomqvist[1], T. Löppönen[2], K. Holli[3], A. Mannermaa[5], J. Kere[4], O.-P. Kallioniemi[3], R. Winqvist[2], H. Nevanlinna[1]*. 1) Dept. of Obstetrics and Gynecology, and Oncology, Helsinki Univ. Centr. Hospital, Finland; 2) Dept. of Clinical Genetics, Univ. of Oulu/Oulu Univ. Hospital, Finland; 3) Lab. of Cancer Genetics, Tampere Univ. Hospital, Finland, and Cancer Genetics Branch, NHGRI, NIH, USA; 4) Dept. of Medical Genetics and Finnish Genome Centre, Univ. of Helsinki, Finland; 5) Unit of Clinical Genetics of Dept. of Gynecology, Kuopio Univ. Hospital/Univ. of Kuopio, Finland.

The result of mutation analyses on the two major breast/ovarian cancer susceptibility genes, BRCA1 and BRCA2, have shown mutational clustering in the genetically isolated Finland. Haplotype analysis of 5 BRCA1 and 4 BRCA2 mutations recurrently found in our population revealed that all families carrying the same alteration have a common ancestor (about 18-80 generations ago, depending on the mutation). Some of the recurrent mutations have also been seen elsewhere in the world, while others are exclusively of Finnish origin. The clustering of the mutations in certain geographical regions gives us remarkable diagnostic advantages, enabeling targeted genetic tests for families from different parts of the country. In addition, almost all BRCA1 and BRCA2 families identified in Finland so far were included in the genotype-phenotype analysis, the results of which in large confirmed the previous observations of preferentially different phenotypic expression of mutations of the 5' and 3' ends of BRCA1, and the earlier onset of ovarian cancer in the families with BRCA1 mutations. In the present study the boundary for differential expression of the BRCA1 mutations in mainly ovarian or breast cancer appeared to be between exons 11 and 12. L. S. and P. H. contributed equally to this study, and H. N. and R. W. share the senior authorship.

Program Nr: 1803 from the 1999 ASHG Annual Meeting

Complete p53 mutation analysis, allelic loss and microsatellite instability in non-small cell lung cancer. *E. Saunders, R. Shipman.* Visible Genetics Inc., Toronto, Ontario, CANADA.

p53 gene mutations in primary cancers may have value as markers for treatment response and outcome. A protocol has been developed which permits routine mutation analysis of the entire p53 gene (exons 2-11) from genomic DNA in both clinical and archival specimens. 67% (18/27) of the primary, non-small cell lung cancers (NSCLCs) examined in this study harboured p53 gene mutations. Molecular genetic studies of primary NSCLC have identified regions of invariant allelic loss at many chromosomal loci. 63% (15/24) of the NSCLCs displayed allelic loss at one or more of the loci examined in this study. The frequent allelic deletion of these discrete chromosomal loci suggests that loss of genes within these regions may influence the development and progression of primary NSCLC. Microsatellite instability (MSI) has also been observed in NSCLC but the frequency of MSI is variable amongst the reported studies. 13% (3/24) of the NSCLCs in this study displayed MSI at one or more of the loci examined. The molecular genetic defects responsible for this instability may play a significant role in the development of some NSCLCs. In addition p53 mutations, allelic loss and MSI were examined for association with clinical and histopathological parameters including treatment response and survival.

Program Nr: 1804 from the 1999 ASHG Annual Meeting

Using Comparative Genomic Hybridization Data to Infer Tree Models of Oncogenesis. *A.A. Schaffer[1], R. Desper[2], F. Jiang[3], O.-P. Kallioniemi[4], C.H. Papadimitriou[5]*. 1) NCBI/NIH, Bethesda, MD; 2) Deutsches Krebsforschungzentrum, Heidelberg, Germany; 3) Institute of Pathology University of Basel, Basel, Switzerland; 4) Cancer Genetics Branch, NHGRI/NIH, Bethesda, MD; 5) Department of Elctrical Engineering and Computer Science, U. C. Berkeley, Berkeley, CA.

Comparative genome hybridization (CGH) is a laboratory method to measure gains and losses of chromosomal regions in tumor cells. It is believed that DNA gains and losses in tumor cells do not occur entirely at random, but partly through some flow of causality. Models that relate tumor progression to the occurrence of DNA gains and losses could be very useful in hunting cancer genes and in cancer diagnosis. We lay some mathematical foundations for inferring a model of tumor progression from a CGH data set. We consider a class of tree models that are more general than a path model that has been developed for colorectal cancer. We derive two tree model inference algorithms based on the ideas of (1) maximum-weight branching in a graph and (2) fitting a matrix of distances to a phylogenetic tree. We prove that under plausible assumptions our algorithms infer good trees. We have implemented our methods in software, and we illustrate with a CGH data set for renal cancer. Our tree models confirm the know role of losses on 3p, and suggest an important role for losses on 4q in renal cell carcinoma.

Program Nr: 1805 from the 1999 ASHG Annual Meeting

Frequency of *BRCA* mutations among a series of Ashkenazi Jewish men diagnosed with breast cancer. *L. Scheuer[1], M. Robson[1], H. Hampel[2], P. Borgen[1], L. Norton[1], D. McDermott[1], M. Pinto[1], J. Hull[1], R. Baum[1], K. Nafa[1], N. Ellis[1], A. Schluger[1], K. Offit[1]*. 1) Department of Human Genetics, Memorial Sloan-Kettering Cancer Center, New York, NY; 2) Arthur G. James Cancer Hospital and Research Institute, Ohio State University, Columbus, OH.

Emphasis has been focused on determining the frequency of three *BRCA* founder mutations (*BRCA1* 185delAG and 5382insC, *BRCA2* 6174delT) in Ashkenazi women with breast and/or ovarian cancer. The frequency of these three mutations in Ashkenazi males with breast cancer has not previously been examined. With informed consent, blood samples were collected from sixteen males at Memorial Sloan-Kettering Cancer Center diagnosed with breast cancer who identified themselves as being at least of 1/4 Jewish descent. All cases were analyzed for the presence of the three *BRCA* founder mutations listed above. In addition, full *BRCA2* sequencing was performed on eleven of the specimens. Six of the sixteen male breast cancer cases (37.5%) were observed to carry one of the three truncating mutations in *BRCA1* or *BRCA2*. Five of the participants had the *BRCA2* mutation, 6174delT and one had the *BRCA1* mutation, 185delAG. In addition, two of the eleven cases that underwent *BRCA2* sequencing (18.2%) were found to have genetic variants of uncertain significance. The mean age of diagnosis of participants with deleterious mutations (60.3 years) was not significantly different from that of noncarriers (59.4 years). Carriers were more likely to report a family history of breast or ovarian cancer in a maternal first-degree relative or a paternal first-or second-degree relative (83.3%) than noncarriers or those with genetic variants (50%). Our findings suggest a high frequency of *BRCA* mutations in Ashkenazi Jewish males diagnosed with breast cancer and provides a rationale for *BRCA1* and *BRCA2* gene testing in all Ashkenazi men with breast cancer, regardless of age of diagnosis and family history.

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Program Nr: 1806 from the 1999 ASHG Annual Meeting

Novel alternatively spliced isoforms of the Neurofibromatosis 2 tumor suppressor demonstrate tissue-specific expression pattern and distinct subcellular localization. *B. Schmucker[1], Y. Tang[1], M. Kressel[2]*. 1) Inst of Human Genetics, Univ Erlangen, Erlangen, Germany; 2) Institute of Anatomy, Univ Erlangen, Erlangen, Germany.

The NF2 protein, merlin, belongs to the protein 4.1 superfamily, a group of closely related membrane-cytoskeleton linkers that regulate cell adhesion and cortical morphogenesis. So far, merlin and the recently cloned DAL-1 are the only members of the superfamily protein 4.1 to act as tumor suppressors. The functional diversity of the prototypical protein 4.1 has been shown to be due to post-translational modifications, usage of two translation initiation sites and an all above extraordinarily complex pattern of alternative splicing. As shown for other tumor suppressor genes as NF1, APC and WT1 tissue integrity appears to be dependent of the relative expression level of different alternatively spliced isoforms. Therefore, we looked at the expression of different splice variants of the NF2 gene in human adult and fetal cDNA panels as well as in non-tumor and tumor cell lines. Three novel full-length isoforms have been identified resulting in altered amino acid sequences. Database search of these amino acid sequences revealed no significant homology to other proteins. Additionally usage of cryptic splice sites has been identified. Furthermore RT-PCR analyses showed tissue- and development-specific expression of novel NF2 transcripts. Evidence for a stable expression of these isoforms on the protein level was found by western blot analyses using different antibodies. To examine the biological significance of these novel isoforms, we expressed epitope-tagged wild type NF2 and splice variants in NIH3T3 cells by using both nuclear microinjection and lipid mediated transfection. Transfection experiments and subsequent confocal laser scanning microscopy revealed targeting of the novel protein isoforms to yet unrecognized subcellular compartments pointing towards functional diversity of the NF2 isoforms. Supported by DFG grant.

Program Nr: 1807 from the 1999 ASHG Annual Meeting

Clonality studies in giant cell tumor of bone. *H.S. Schwartz[1], J.D. Eskew[2], M.G. Butler[2]*. 1) Orthopaedics & Rehabilitation , Vanderbilt Univ Medical Ctr, Nashville, TN; 2) Section of Medical Genetics and Molecular Medicine, The Children's Mercy Hospitals and Clinics, Kansas City, MO.

Giant cell tumor of bone (GCT) is a solid, primary bone tumor composed of stromal mononuclear cells and large multinucleated cells which resemble osteoclasts. It represents 5% of skeletal neoplasms and has an unpredictable pattern of biological aggressiveness manifesting as local recurrences and infrequent benign pulmonary metastasis. Cytogenetic studies have demonstrated telomeric associations and other chromosome abnormalities, specifically rings and markers, in as many as 50% of tumor cells in the majority of patients. Telomerase activity and telomere reduction have been demonstrated in tumor cells from GCT patients. In addition, microsatellite instability and loss of heterozygosity (LOH) studies have been performed on cells from GCT patients using several polymorphic DNA markers and no instability or LOH were found. To assess whether the population of GCT cells is monoclonal or polyclonal in proliferation, DNA was isolated from fresh frozen tumor and normal tissue from GCT individuals. A polymerase chain reaction-based assay for methylation of the polymorphic X-linked human androgen receptor gene (HUMARA) was used to assess clonality following established protocols from other tumor studies. To date, we have analyzed six female GCT subjects ranging in age from 20 to 43 years and the androgen receptor gene data supports polyclonal proliferation in all cases. Polyclonal proliferation has also been found in giant cell tumor of tendon sheath which is a common soft tissue tumor of synovial cell origin. Our studies suggest that giant cell tumor of bone is also polyclonal and not neoplastic if one assumes that cells forming a neoplastic mass must demonstrate monoclonality to be classified as a neoplasm.

Program Nr: 1808 from the 1999 ASHG Annual Meeting

Human acid ceramidase located at 8p22 is overexpressed but not mutated in prostate cancer. *R.S. Seelan, C. Qian, A. Yokomizo, D.I. Smith, W. Liu.* Div. of Experimental Pathology, Mayo Clinic & Foundation, Rochester, MN.

The 8p22 region of the human chromosome is frequently deleted in a variety of tumors including prostate cancer, suggesting that a putative tumor suppressor gene (or genes) is located within this region. The human acid ceramidase gene, which causes Farber disease, is located in this region between microsatellite markers D8S258 and D8S261 based on overlapping YAC contigs that span these markers. Ceramidase catalyzes the hydrolysis of ceramide, a potent lipid second messenger that promotes apoptosis and inhibits cellular proliferation. The chromosomal localization and the functional characteristics of this gene make it a promising tumor suppressor for prostate cancer. We describe here the structural organization of the gene and its 5' flanking (putative promoter) region. This gene contains 14 exons spanning about 28 kb of the genome. Comparison of the promoter sequences between the mouse and human ceramidase genes suggests that these two genes diverged considerably during evolution. Northern blot analysis indicates that ceramidase is highly expressed in prostate and blood but poorly expressed in testis and thymus. Quantitative RT-PCR analysis indicates that this gene is overexpressed in prostate cancer tissue, especially in high-grade tumors, compared with matched normals. Mutation analysis of this gene by Southern blot analysis and Denaturing HPLC in 28 prostate cancer and 12 sets of matched normal/prostate tumor samples revealed no mutations except for 12 single nucleotide polymorphisms (SNPs). Two of them are coding SNPs, in exons 3 and 4, and 7 of them are clustered in exon 14 within a 142 bp stretch. Although these findings rule out a tumor suppressor role for this gene in prostate cancer, it is possible that other types of mutations in the non-coding region might exist. The information provided, herein, should prove useful for large-scale screening of patients suffering from Farber disease or from disorders resulting from altered ceramide levels.

Program Nr: 1809 from the 1999 ASHG Annual Meeting

Mosaicism in von Hippel-Lindau Disease: Two Kindreds Each with an Affected Mosaic Parent Whose Offspring Carry Germline Mutations. *M.T. Sgambati[1], C. Stolle[2], P.L. Choyke[3], M.M. Walther[1], B. Zbar[1], W.M. Linehan[1], G.M. Glenn[1]*. 1) National Cancer Institute,Bethesda MD; 2) Genetic Diagnostics Laboratory, University of Pennsylvania, Philadelphia, PA; 3) National Institutes of Health, Bethesda, MD.

VHL (MM# 193300)is a heritable autosomal dominant multiple neoplastic disorder characterized by brain and spinal cord hemangioblastomas, retinal angiomas, clear cell renal carcinoma, neuroendocrine tumors and cysts of the pancreas, pheochromocytomas, endolymphatic sac tumors, and papillary cystadenomas of the epididymis and broad ligament. While most index cases have a positive family history of VHL, some do not. Cases without a family history of VHL may or may not have a germline mutation in their VHL tumor suppressor gene. We present here two cases of VHL mosaicism. In each of two families, standard-testing methods (Southern Blot and direct sequencing) identified the germline mutation in the VHL gene of the offspring, but not in their clinically affected parent. Further analyses of the affected parents' blood detected the VHL gene mutation in a portion of their peripheral blood lymphocytes. Detection of the deleted allele in one case was by fluorescence in situ hybridization (FISH), and in the second case,the three base pair deletion was detected by conformational sensitive gel electrophoresis (CSGE) and DNA sequencing of cloned genomic DNA. Mosaicism in VHL is important to search for and recognize. Identification of mosaic individuals has implications for counseling families and screening for disease in the mosaic individual.

Program Nr: 1810 from the 1999 ASHG Annual Meeting

Prompt improvement of acute promyelocytic leukemia (FAB M3) by classic therapeutic regimen in a patient without chromosomal abnormality, yet exhibiting PML/RARA translocation by fluorescence in situ hybridization. *H.O. Shah[1, 2], J.H. Lin[1, 2], M. Chester[1], W. Aung[1], J. Siddiqui[1], M. Garrison[1, 2].* 1) Nassau County Medical Ctr, East Meadow, NY; 2) State University of New York SUNY, Stony Brook, NY.

This 25 year old Hispanic woman treated for an acute orbital cellulitis for two days. Laboratory tests showed WBC of 1.8k/mm3, RBC of 2.64 mil/mm3, Hb of 8.8 gm/dl, Hct 25%, and platelet count of 25k/mm3. The peripheral blood revealed 70% of myeloblasts and promyelocytes. Many promyelocytes were reniform and some contained coarse granules and/or Auers rods. The aPTT and PT were elevated; fibrinogen was 122 mg/dl. The marrow cellular morphology disclosed acute promyelocytic leukemia (FAB M3), yet the marrow chromosomal study expressed 46,XX with no structural abnormality. Fluorescence in situ hybridization studies revealed specific translocation i.e. t(15;17)(q22;q11) in 370 of 500 interphase cells, i.e. 46,XX. nuc ish 15q22 (PMLx2),17q12(RARAx2)(RARA con PMLx1) and the rest of the cells revealed a normal signal pattern nuc ish 15q22(PMLx2),17q11(RARAx2) negative for presence of clone with PML/RARA rearrangement by interphase. The patient was then treated with ALL Trans Retionic Acid (ATRA). The coagulation parameters improved immediately and returned to normal in seven days. On the 13th ATRA therapy day, the WBC was 13.0k/mm3 with 5% myeloblasts, 12% promyelocytes, and the chromosomal make-up remained 46,XX. Because of good clinical response, she was continued with ATRA. On the 20th day she was started with induction therapy with Daunorubicin and Cytosin Arabinoside. Additional three weeks later she was in complete hematological remission and recovery from the periorbital lesion. She then received two additional cycles of chemotherapy and remained in complete remission ten months after diagnosis clinically and molecular-cytogenetically.

Program Nr: 1811 from the 1999 ASHG Annual Meeting

Uptake of Free BRCA1/2 Testing in Women at Risk for BRCA1/2 Germline Mutations. *K.M. Shannon[1], M.L. Lubratovich[1], D. Finkelstein[2], W. Goggins[2], D.A. Haber[1], M.V. Seiden[1].* 1) Dept Hematology/Oncology, Massachusetts General Hosp, Boston, MA; 2) Dept Biostatistics, Massachusetts General Hosp, Boston, MA.

Barriers to genetic testing for BRCA1/2 mutations have been examined in various contexts. Cost of gene sequencing has been identified as a possible deterrent, given that many women would prefer to not involve their insurance companies and/or their insurer will not reimburse for testing. The recent availability of a novel BRCA1/2 detection system at our institution has allowed us to evaluate this question directly. From 4/97-1/99, 47 women undergoing genetic counseling at our institution were identified as having a family history suggestive of an altered BRCA1/2 gene. The women were offered genetic testing and declined, citing high cost as the primary factor. All indicated they would be interested if the cost were greatly reduced. In 1/99, the women were invited to participate in a research study through which BRCA1/2 testing would be offered at no cost. They were asked to return a survey and indicate if they remained interested in testing. 30 responded yes, 2 responded no and 15 did not return the survey. Of the 30 who responded yes, 2 declined prior to scheduling a pre-test education visit and 2 declined after pre-test education. We were interested in factors that may affect decisions to pursue (N=26) or not pursue (N=6)free testing. Probabilities of carrying a mutation in either BRCA1 or BRCA2 were calculated using BRCAPRO software. The mean risk for women who declined testing (0.54) was significantly higher than those who desired testing (0.21) with P=.025. Examination of other variables including elapsed time between the initial genetic counseling session, whether or not the woman had sisters or female children demonstrated no significant correlation with decision to pursue testing. The association between testing decision and having children was marginally significant (p=.10). Our study suggests that in a group of women who cite the high cost of BRCA1/2 testing as the primary deterrent to testing, removing this barrier prompts testing in only half the group. Further study of a larger cohort is required to identify other barriers to testing in this patient population.

Program Nr: 1812 from the 1999 ASHG Annual Meeting

Chromosome 7q22 is a likely site of a Tumor Suppressor Gene in Malignant Myeloid Diseases. *V. Shashi[1], M.J Pettenati[1], C. von Kap-Herr[1], D.W Bowden[2].* 1) Pediatrics; 2) Biochemistry, Wake Forest Univ Sch Med, Winston-Salem, NC.

Monosomy of chromosome 7 or partial deletion of 7q is seen in 10% of Myelodysplastic syndromes (MDS) and Acute Myeloid Leukemia (AML) and in as many as 50% of therapy related MDS/AML. Previous studies using FISH have reported 7q22, 7q32-q33 and 7q35-q36 as commonly deleted regions. This has led to the hypothesis that there are one or more tumor suppressor genes on 7q responsible for MDS/AML A specific tumor suppressor gene has not yet been identified.

We have performed FISH on 21 consecutive cases of MDS/AML cases with a cytogenetic deletion of 7q. Interstitial (n=11) and terminal deletions (n=10) occur equally frequently, as determined by FISH with the 7q telomere specific probe. Thirteen YACs spanning the long arm of chromosome 7 from band q11.23-q36 were used to further delineate the deletions. Terminal deletions are clustered proximally between q11.23-q22. In interstitial deletions, proximal breakpoints mostly occur at 7q22 (n=8/11). The distal breakpoints vary from 7q31.1-q35. Bands 7q22 and q31.1 are the most commonly deleted segments in both terminal and interstitial deletions. Unlike previous studies we did not find a significant number of distal deletions involving 7q35-36.

Our results indicate that terminal and interstitial 7q deletions occur roughly equally in MDS/AML. Although there is heterogeneity in breakpoints in 7q deletions there is a commonly deleted area involving 7q22 and 7q31.1 in both interstitial and terminal deletions. Further deletion mapping of the 7q22-q31.1 region by FISH using a YAC contig will be helpful in determining the location of a putative tumor suppressor gene.

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Program Nr: 1813 from the 1999 ASHG Annual Meeting

Application of molecular cytogenetic analysis for clarification of unusual chromosome rearrangements in a child with acute myelogenous leukemia (AML). *S. Shekhter-Levin[1], S. Orlando[2], L.C. Contis[3], M.E. Sherer[1], X. Huang[1], S. Stanke[5], S.M. Gollin[1,2,4]*. 1) Human Genetics, Univ Pittsburgh Graduate School of Public Health, Pittsburgh, PA; 2) Pediatrics, Univ Pittsburgh School of Medicine; 3) Pathology, Univ Pittsburgh School of Medicine; 4) Univ Pittsburgh Cancer Institute; 5) Applied Imaging, Santa Clara, CA.

The patient is a 2-year-old female with a one week history of fever, leukocytosis, anemia, thrombocytopenia and blasts in her peripheral blood. Peripheral blood and bone marrow pathology studies, including flow cytometry, supported the diagnosis of AML, most likely of the M5a FAB subtype. Classical cytogenetic analysis of G-banded metaphase cells from unstimulated bone marrow aspirate cell cultures at diagnosis revealed the following mosaic abnormal karyotype: 46,XX,?add(8)(p11.2),inv(9)(p11q13),+?19,-?22[7]/46,idem,?add(17)(q25)[12]. The inv(9)(p11q13) is considered a common constitional variant. The interpretation of the other findings was unclear, but was essentially solved using molecular cytogenetics. FISH studies with the BCR/ABL, MYC, and chromosome 17 alpha-satellite (D17Z1) DNA probes showed that the additional 19 is an i(22)(q10), the ?add(8)(p11.2) carries MYC, and the ?add(17)(q25) contains the chromosome 17 centromere. The RxFISH multicolor banding study revealed that the add(8)(p11.2) appears to be the result of a pericentric inversion, inv (8)(p11.2q21.2) and the add(17)(q25) is most likely an unbalanced translocation with chromosome 6p, der(17)t(6;17)(p21.1;q25). The latter was confirmed with chromosome painting with the WCP6 probe combined with the chromosome 17 alpha-satellite DNA probe. As a result of the FISH studies, the final karyotype was revised as follows: 46,XX,inv(8)(p11.2q21.2),inv(9)(p11q13),i(22)(q10)[7]/46,idem,der(17)t(6;17)(p21.1;q25)[12]. ish inv(8)(p11.2q21.2)(MYCx1), der(17)t(6;17)(p21.1;q25)(wcp6+;D17Z1+), i(22)(q10)(BCRx2). Thus, combining classical chromosome analysis with molecular cytogenetics, including the new multicolor banding method, RxFISH, significantly clarified our understanding of the unusual acquired chromosome abnormalities in this child with AML.

Program Nr: 1814 from the 1999 ASHG Annual Meeting

Investigating the role of the RET proto-oncogene in small cell lung carcinoma (SCLC). *Y. Shi, B. Campling, S. Myers, L. Mulligan.* Pathology, Queen's University, Kingston, Ontario, Canada.

SCLC clinically often presents many neuroendocrine features. The RET proto-oncogene encodes a receptor tyrosine kinase, which is expressed in cells derived from the neural crest. Mutations of RET have been implicated in neuroendocrine cancers including medullary thyroid carcinoma, and in the cancer syndrome multiple endocrine neoplasia type 2. However, we have shown that no mutations occur in SCLC. RET is normally activated by binding to both a ligand of the glial cell line-derived neurotrophic factor family (GDNF, NTN), and a GPI-linked coreceptor the GFR family (GFRa-1, 2, 3). Our previous studies have shown that RET, GDNF, and GFRa-1 transcripts are expressed in some SCLC cell lines. Thus, we predicted that paracrine or autocrine activation of RET could occur in these lines, and might play an important role in SCLC growth or differentiation. Using 3 cell lines selected for the expression of RET, GDNF, and GFRa-1 transcripts, we have detected RET protein expression in 2 cell lines (MOA, YRA) by immunoprecipitation and western-blotting of RET protein. We have also found cell surface GFRa-1 protein expression in these cell lines by flow cytometry. Thus, these lines have the potiential to respond to GDNF. To address the possibility that other RET ligands (NTN) or coreceptors (GFRa-2 or 3) might be involved in RET activation, we screened for NTN, GFRa-2, and GFRa-3 transcripts by RT-PCR in 22 SCLC cell lines. We have shown that 50% (11/22) of cell lines express NTN. Further, 72% (16/22) of lines express GFRa-3 transcripts, but the expression of GFRa-2 occurs in only a subset of these lines. Our data suggest that SCLC cell lines could potentially be responsive to RET activation through one or more of the possible ligand-receptor complexes. We are currently investigating the response of SCLC to RET ligands using assays for RET phosphorylation and for morphological changes in these cell lines. Our study will elucidate the role of RET in the growth regulation of SCLC.

Program Nr: 1815 from the 1999 ASHG Annual Meeting

Cloning and characterization of genes at reciprocal translocation t(1;9)(p32.3;p21.2) breakpoint in a patient with neuroblastoma. *H.O. Shiwaku[1], M. Hoshi[1], H. Tsuchiya[2], I. Kamino[3], Y. Kaneko[4], A. Horii[1]*. 1) Department of Molecular Pathology, Tohoku University School of Medicine, Sendai, Miyagi, Japan; 2) Department of Pediatrics, Kumamoto University School of Medicine, Kumamoto, Japan; 3) Special Reference Laboratory, Hachioji, Tokyo, Japan; 4) Department of Cancer Chemotherapy, Saitama Cancer Center, Saitama, Japan.

Neuroblastoma is a well-known malignant disease in infants, but its molecular mechanisms have not yet been elucidated. The most frequently observed chromosomal aberration is loss of chromosome arm 1p in neuroblastomas, and this chromosome arm may harbor two or more tumor suppressor genes in this disease. We have established a cell line that was derived from lymphoblast of a patient with neuroblastoma. This patient has a constitutional reciprocal translocation t(1;9)(p32.3;p21.2). A contig spanning an approximately 800-kb consisting of ten BAC clones of minimal overlap was constructed and three overlapping clones that harbor the breakpoint were cloned. A cosmid library was also constructed with the DNA of the patients cell line and the translocation breakpoint was further characterized in detail. We also performed cDNA screening and detected two transcripts from this region. Results of the characterization of the breakpoint as well as cDNA clones will be described in detail.

Program Nr: 1816 from the 1999 ASHG Annual Meeting

The importance of cytogenetic analysis in the diagnosis and treatment of bone and soft tissue sarcoma. *R. Shomrat[1], Y. Yaron[1], M. Goldstein[1], D. Kunig[1], J. Issakov[3], I. Bar-Am[4], I. Meller[2], A. Orr-Urtreger[1].* 1) Genetic Inst, Tel-Aviv Sourasky Med Ctr, Tel-Aviv, Israel; 2) The national unit of Orthopedic OncologyTel-Aviv Sourasky Med Ctr, Tel-Aviv, Israel; 3) Pathology Inst,Tel-Aviv Sourasky Med Ctr, Tel-Aviv, Israel; 4) Applied Spectra Imeging Ltd., Israel.

Chromosomal analysis is becoming increasingly useful in the diagnosis and management of bone and soft tissue sarcomas. The identification of chromosomal aberrations, such as translocations, deletions and/or additions of a part or whole chromosome, double minutes (dmin), and other markers are associated with specific tumor subtypes. We have analysed 78 samples of tumors. The cytogenetic analysis was carried out on a short-term cultured tissues by G-banding, FISH and SKY procedures, as needed. (*)Histopathological examination excluded malignancy in 19 out of the 78 samples. The remaining 59 tumors were diagnosed as Osteosarcoma (16), Ewing's sarcoma (13), Synovial Sarcoma (4), Rhabdomyosarcomas (1 embryonal and 3 alveolar), 3 Liposarcomas (1 myxoid), 3 Extraabdominal Fibromatosis (Desmoid Tumors), and 16 other different types sarcoma. (*)Among the 16 Osteosarcomas studied 8 have demonstrated complex hyperploid karyotypes compatible with the diagnosis of high grade osteosarcoma. In most of the Ewing's sarcomas (including 3 with the typical t(11;22) translocation) other chromosomal abnormalities were seen like trisomies of chromosomes 5, 6, 8 and 14. Three of the four Synovial sarcomas had the typical t(X;18)(p11.2;q11.2) translocation. One of the synovial sarcomas was intially diagnosed histopathologically as Ewing's sarcoma, but the cytogenetic analysis demonstrated a complex X;18 translocation and led to diagnostic revision and specific treatment. Only one of the Alveolar Rhabdomyosarcomas demonstrated the typical t(2;13)(q35;q14) translocation, while hypertetraploid set with dmins was detected in the other two cases. By using SKY, we determined that chromosome 1 is the origion of one of the dims, suggesting that PAX7 amplification could be involved in the pathogenesis of this tumor. (*)Cytogenetic analyses of bone and soft tissue tumors are of important clinical value for accurate definition of the tumor type.

Program Nr: 1817 from the 1999 ASHG Annual Meeting

Loss of Expression By Deletion And Hypermethylation of a New Member of the DNAJ Protein Family On 13q14.1 in Ovarian Cancer. *V. Shridhar[1], J. Staub[1], H. Huang[1], G. Callahan[1], R.K. Bright[2], A. Yokomizo[1], L. Wang[1], H.I. Pass[2], L. Hartmann[1], D.I. Smith[1]*. 1) Experimental Pathology, The Mayo Clinic, Rochester, MN; 2) Department of Surgery, Karmanos Cancer Institute, Wayne State University, Detroit, MI.

We isolated a differentially expressed transcript and its corresponding gene by differential display PCR between a prostate tumor cell line and a cell line derived from matched normal prostate epithelium [1542NPTX and CPTX]. Sequencing followed by EST-based walking of this fragment yielded a new full length cDNA which could code for a predicted 150 amino acid long protein with a transmembrane domain at its N terminus and a DNAJ domain at its C terminus. The gene which mapped to 13q14.1 was designated as MCJ (methylation controlled J protein)and has partial homology in the DNAJ domain to a number of proteins with a similar domain. The genomic structure of MCJ revealed that it contains four exons. MCJ has the highest similarity to a functionally undefined protein from C.elegans. MCJ is expressed as a 1.2Kb transcript in several tissues with testis showing the highest level of expression. Expression of MCJ was completely absent in a cell line established from benign prostate hyperplastic epithelium (the BPH-1 cell line), three of eight ovarian cancer cell lines, in 5 of 28 primary ovarian tumors and three of eight mesothelioma cell lines. Expression was induced in a dose dependant manner in the BPH-1 cell line by 5-aza-2'-deoxycytidine (5-aza-2'-dC) treatment implicating the involvement of methylation in this induction. Expression analysis using semi-quantitative RT-PCR indicates that more than 66% (12/18) of primary ovarian tumors had either a complete absence and /or lower levels of expression of this gene. While no tumor specific mutations were detected in any of the coding exons in cell lines and primary tumors, LOH analysis with a new microsatellite marker identified 80 bases 3' to the gene and Methylation Specific PCR analysis revealed that in primary tumors and cell lines with loss of MCJ expression showed that one allele was lost by methylation and the other by deletion.

Program Nr: 1818 from the 1999 ASHG Annual Meeting

A Genetic study of Hodgkin's lymphoma: an estimate of heritability and anticipation using the Familial Cancer Database in Sweden. *Y.Y Shugart[1], K. Hemminki[2], P. Vaittinen[5], C. Dong[2], J.R. O'Connell[4], A. Kingman[3].* 1) Pediatrics/CIDR, Johns Hopkins University, Baltimore, MD; 2) Department of Biosciences, Karolinska Institute, Huddinge Sweden; 3) National Dental and Craniofacial Institute, Bethesda, MD; 4) Department of Human Genetics, University of Pittsburgh, Pittsburgh PA; 5) Center for Epidemiology, National Board for Health and Welfare, Stockholm Sweden.

Genetic anticipation in Hodgkin's lymphoma (HL) has been reported by Shugart (1998). However, the previous study uses data from published literature and contains a number of biases. We are interested in testing the hypothesis of anticipation in the Swedish population because it is feasible to search for independently ascertained affected relatives through the Familial Cancer Database in Sweden. By linking two Swedish registries: a second generation registry maintained by Statistics Sweden and the Cancer Registry maintained at the Center for Epidemiolgy, we identified 12 parent-child pairs who were both affected with HL. The usual t-test method was used to test whether there is a difference in cancer age of onset between parents and children who are affected with HL. A randomization test was used to test the validity of the p-values. The mean age of onset is 38.41 years (SD=14.61) for parents and 25.58 years (SD=10.91) for the children. The mean anticipation is 12.8 years (SD=11.2). The corresponding paired t-test statistic is 3.98. Assuming normality it would have 11 degrees of freedom with an associated p=0.0011 (one sided). This finding is unlikely to be explained by sampling bias alone. Using methods proposed by Falconer and Mackay (1997), we also estimated the heritability of HL to be 28.4% in the Swedish population.

Program Nr: 1819 from the 1999 ASHG Annual Meeting

Familial Cancer Database. *R.H. Sijmons[1], G.T.N. Burger[2].* 1) Medical Genetics, Univ Groningen, Groningen, Netherlands; 2) Sazinon Foundation, Bethesda Hospital, Hoogeveen, Netherlands.

Cancer is associated with a wide range of hereditary disorders. Recognizing these disorders in cancer patients may be of great importance for the medical management of patients as well as their relatives. Conversely, recognizing the fact that cancer may develop in patients already diagnosed with a hereditary disorder may be important for the same reason. We have developed a stand-alone interactive computer program, Familial Cancer Database, to assist the clinician and the genetic counselor in making a genetic differential diagnosis in cancer patients as well as in becoming aware of the tumour spectrum associated with a particular hereditary disorder already diagnosed. The program tries to match tumour and non-tumour features observed in patients and their families with any of the more than 300 disorders presently contained in the database and provides a clinical synopsis for each of these disorders. Additional features of the program are to search the more than 1000 references in the database, and to search for disorders associated with particular chromosomal regions and genes. Familial Cancer Database has been tested by an International panel of oncologists and geneticists and will be offered free of charge to all interested physicians and genetic counselors.

Program Nr: 1820 from the 1999 ASHG Annual Meeting

LOW PROPORTION OF BRCA1 AND BRCA2 MUTATIONS IN FRENCH CANADIAN BREAST CANCER FAMILIES. *J.R. Simard[1], F. Durocher[2], H. Vezina[3], C. Samson[1], M. Dumont[1], M. Desrochers[1], N. Bolduc[1], H. Malouin[1], V. Paquet[1], L. Larouche[1], M. Tranchant[1], M. Jomphe[3], J. Chiquette[4], L. Provencher[4], M. Plante[1], R. Laframboise[1], F. Labrie[1], D. Easton[2], P. Bridge[5]*. 1) Laval University Medical Center, Quebec City, Quebec, Canada; 2) CRC Genetic Epidemiology Unit, University of Cambridge,Cambridge, UK; 3) Universite du Quebec Chicoutimi/IREP, Chicoutimi, Quebec,Canada; 4) Hopital St-Sacrement, Quebec City, Quebec, Canada; 5) Alberta Childrens Hospital, University of Calgary, Calgary, Alberta, Canada.

Our study was designed to estimate the frequency and penetrance of mutations in BRCA1 and BRCA2, as well as , their relative contribution and the role of the founder effect and demographic history of the country in the origin and spreading of mutated BRCA1 and BRCA2 alleles. 112 affected women from 61 French Canadian families, which included, at least 4 cases of breast/ovarian cancer, was first tested for the 9 BRCA1 and BRCA2 mutations previously reported in this population. This analysis led to the detection of the R1443X (BRCA1) in 8 families and 8765delAG (BRCA2) in 7 families and 2816insA (BRCA2) in 1 family. Therafter, the complete sequence of all exons and splicing sites of BRCA1 and BRCA2 was done, leading to the detection of 4 novel frameshift mutations and 14 sequence variants. Additional analyses were done to test for large duplication or deletion and regulatory mutation in mutation-negative families. The average age at diagnosis of breast cancer was older in the mutation-negative families (51.8 compared to 45.6 $p<0.001$). Among the 42 mutation-negative families, 35 were site-specific breast cancer families, while only one of these families shows >1 case of ovarian cancer. Genotyping analyses have shown evidence for a founder effect for the two most frequent mutations. Calculation of the mean genetic contribution of the ancestors found to be common to the 8 R1443X families suggest that the two most frequent and specific ancestors married in the Quebec City area. The low frequency of deleterious mutations in the BRCA1 and BRCA2 genes in French Canadian breast cancer families suggests that additional cancer susceptibility genes are likely to be important in this population.

Program Nr: 1821 from the 1999 ASHG Annual Meeting

Silencing of the retinoic acid receptor beta (RAR beta) gene by hypermethylation in human breast cancer. *S. Sirchia[1], A.T. Ferguson[2], S. Sukumar[2], R. Orlandi[3], N. Sacchi[1,2].* 1) School of Medicine,University of Milan, Milan, Italy; 2) Oncology Center, John Hopkins University, Baltimore, MD; 3) Istituto Nazionale Tumori, Milan, Italy.

CpG island hypermethylation is known to be associated with gene silencing in human cancer. It has been proposed that this epigenetic event may be determined either by aberrant DNA methyltransferase (DNA-Mtase) activity or other cellular factors. Recent evidence seems to indicate that, aside from random DNA-Mtase action, additional cellular factors exist that govern aberrant methylation in breast cancer cells. By using methylation specific PCR (MSP) we have shown that methylation of the retinoic acid receptor beta (RAR beta) promoter region containing the retinoic acid responsive element (RARE) is occurring in a great proportion of breast cancer cell lines and primary breast tumors, but not in normal human mammary epithelial cells (HMEC). Methylation of RAR beta in breast cancer cell lines is associated with loss of expression of the RAR beta gene. The expression can be restored by demethylating agents. Interestingly, we have observed that aberrant methylation of RAR beta is occurring also in normal breast tissue surrounding breast cancer cells, but not in normal lymphonodes. These observations suggest that aberrant methylation of RAR beta, leading to silencing of the RAR beta gene may be an early event in breast carcinogenesis.

Program Nr: 1822 from the 1999 ASHG Annual Meeting

Two rare cases of leukemia cutis and a literature review. *R.A. Sokolic[1], E. Sotomayor[2], K. Santoro[2], S. Mark[3], P. Rintels[2], H.F.L. Mark[2,4,5]*. 1) Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY; 2) Rhode Island Hospital, Providence, RI; 3) Brown University, Providence, RI; 4) KRAM Corporation (Medical Genetics Division), Barrington, RI; 5) Brown University School of Medicine, Providence, RI.

Leukemia cutis is a rare presentation of leukemia. It has been reported with many different forms of leukemia, including acute myelogenous leukemia of various subtypes, acute lymphocytic leukemia, B-cell chronic lymphocytic leukemia, and chronic myelomonocytic leukemia. Although, The incidence of this disease varies by leukemic subtype, the morphologic subtype of leukemia seems to have little bearing on the ability of a hematologic malignancy to express itself as leukemia cutis. With the advent of flow cytometry and cytogenetics, hematologic malignancies are more often described as molecular rather than morphologic entities. However, little is known about the molecular cytogenetics of leukemia cutis. The few papers on the cytogenetics of soft tissue lesions in leukemia do not specifically discuss skin lesions. It is not known whether the cytogenetics of leukemia cutis is separate and distinct from the cytogenetics of leukemia cases in general and also from the non-leukemia cutis cases. We report here on the cytogenetic findings in two rare cases of leukemia cutis as a presenting symptom of leukemia in order to shed additional light on the above problem. Results of cytogenetic analyses revealed the following findings: 46,XY,del(9)(q21)[12]/46,XY[10] in patient 1, and 82~91,XXXX,+6,add(7)p22),-10,-10,del(11)(q22)x2,i(17)(q10),+mar[cp35]/46,XX, add(7)(p22),i(17)(q10),+mar[2]/46,XX[1] in patient 2. The existing literature on leukemia cutis was reviewed to establish whether these are recurrent findings.

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Program Nr: 1823 from the 1999 ASHG Annual Meeting

Spontaneous mutation in Big Blue transgenic mice: the effects of age, tissue type, genotype, vitamin E, and carcinogenesis. *S.S. Sommer, K.A. Hill, V.L. Buettner, A. Halangoda, M. Kunishige, S.R. Moore.* City of Hope, Beckman Research Institute, Duarte, CA.

The Big Blue system provides a powerful tool to examine spontaneous mutation and aging, cancer, or neurodegenerative disease. Substantial evidence has accumulated that the isolated mutants reflect mouse *in vivo* mutations. Methodological enhancements have increased the efficiency of determining mutation frequence and pattern 8-fold. In the past two years, the lab screened 58 million plaque forming units and sequenced 2,645 mutants (3.1 Mb). An analysis of spontaneous mutations and gender, genetic background, tissue type, and aging was performed. Mutations were examined in whole embryos and seven tissues (forebrain, cerebellum, thymus, heart, liver, adipose tissues, and male germ cells) in post-natal mice (10d, 3, 10, and 25 mo). Two time courses of mutation frequency and one core mutation pattern were identified. The previously observed constancy of mutation frequency at young and mid-adulthood was confirmed. The male germline demonstrated significantly lower mutation frequencies than somatic tissues. Three classes of multiple mutations were described; those occurring in tandem, 2-10 bp and greater than 20 bp apart. Mutations within 5 bp are most likely single mutation events. Tandem-base mutations occurred only in liver and adipose tissues, increased significantly with age in liver, and were largely coordinate GG to TT mutations. Mutation frequency was altered significantly in mice nullizygous for the DNA repair gene ERCC6. Of particular interest, given the relationship between mutation and cancer, is the reduction of mutation frequency under certain circumstances (Vitamin E supplementation, ERCC6 nullizygosity in neurons, and the enhanced expression of SOD1). Six of 11 thymic lymphomas derived from p53-deficient mice showed small increases in mutation frequency and one tumor showed an altered mutation pattern. The results provide a profile of spontaneous mutation from which deviations due to alterations in genotype and mutagen exposure are measured.

Program Nr: 1824 from the 1999 ASHG Annual Meeting

Hypotetraploidy in a patient with small cell carcinoma. *E. Sotomayor[1], B. Hongwei[2,3], S. Mark[4], P. Rintels[2,3], H.F.L. Mark[3,5]*. 1) University of Texas Medical Br, Galveston, TX; 2) Rhode Island Hospital, Providence, RI; 3) Brown University School of Medicine, Providence, RI; 4) Brown University, Providence, RI; 5) KRAM Corporation, Barrington, RI.

While numerical and structural chromosomal abnormalities characterize many hematopoietic and non-hematopoietic malignancies, a review of the literature using MEDLINE, CANCERLIT and the Science Citation Index revealed that the occurrence of polyploidy is by and large rare. We report here an interesting patient with small cell carcinoma (SCC) and polyploidy initially referred to us because of a question of acute nonlymphocytic leukemia (ANLL), M3 subtype, with a question of a 15;17 translocation characteristic of acute promyelocytic leukemia. However, the patient did not have a 15;17 translocation and the final hematopathologic analysis of the bone marrow aspirates and immunohistochemistry studies subsequently revealed the patient to have SCC. Small cell carcinoma is a highly malignant and a very aggressive neoplasm. In leukemia, reports of polyploidy point to a distinct category of patients with a poor risk for which more intensive treatment is needed. Little information is currently available to assess the risk of polyploidy in small cell carcinoma. This case is important because of the relative rarity of polyploidy/tetraploidy. Insights gained from the study of this and other similar patients may also help shed additional light on the mechanism of carcinogenesis, which is not fully known to-date. As polyploidization is a manifestation of genetic instability and as genetic instability has been implicated in the genesis and progression of many cancers, it is perhaps not too surprising that hypotetraploidy in this case was associated with a poor disease outcome, namely the demise of the patient.

Program Nr: 1825 from the 1999 ASHG Annual Meeting

Increased Frequency of Glutathione-S Transferase (GSTT1) Null Genotype in Patients with Aplastic Anemia and Myelodysplastic Syndromes. *M.W. Stacey[1], T. McAninch[1], L. Santi[1], C. Osgood[1], R.L. Byrd[2], J.M. Liu[3], N. Young[3], W.G. Kearns[1,4].* 1) Center for Pediatric Research, Norfolk, VA; 2) Division of Hemotology/Oncology, Children's Hospital of the King's Daughters, Norfolk, VA; 3) Hematology Branch, NHLBI, NIH, Bethesda, MD; 4) Inst. of Genetic Medicine, Johns Hopkins Univ Sch of Med. Baltimore, MD.

Background: Aplastic anemia (AA) is a disorder of hemopoetic bone marrow stem cells and is rapidly fatal if left untreated. Patients receiving immunotherapy are at risk for late complications, including progression to myelodysplastic syndromes (MDS). Toxic environmental factors are postulated to contribute to the etiology of AA and MDS. It is possible that patients with a reduced ability to metabolize carcinogens or toxins are at risk for developing AA with progression to MDS. The glutathione S-transferases, GSTT1 and GSTM1, encode proteins that play a role in metabolizing carcinogens and toxins. **Objective:** To determine the prevalence of GSTT1 and GSTM1 null alleles in bone marrow cells from patients with AA, MDS, and controls. **Methods:** Multiplex PCR was performed on extracted DNA to co-amplify the GSTT1 alleles, the GSTM1 alleles, and -globin or actin as an internal control. **Results:** The GSTT1 null genotype was found in 8/26 (31%) AA patients, 9/15 (60%) MDS patients, and 6/37 (16%) control samples. The GSTM1 null genotype was found in 15/26 (58%) AA and 9/15(60%) MDS patients. Fifty-four percent (20/37) of controls had the GSTM1 null genotype. The incidence of the GSTT1 null genotype was significantly higher ($p<0.05$) for the AA and MDS patients versus controls. No significant difference was found in the frequency of the GSTM1 null genotype between controls and patients. Ethnic variability will be discussed. **Conclusions:** These preliminary results suggest there is a significant increase in the frequency of the GSTT1 null genotype in AA and MDS patients from this studied population pool. Abnormal detoxification of environmental or endogenous toxins might lead to genetic instability with possible disease progression.

Program Nr: 1826 from the 1999 ASHG Annual Meeting

Multiplex RT-PCR screening for the detection of specific fusion-genes in childhood leukemia. *S. Strehl[1], H. Christiansen[2], J. Lovmand[2], O.A. Haas[1]*. 1) CCRI, Children's Cancer Research Institute, Vienna, Austria; 2) DNA Technology, A/S, Science Park Århus, Denmark.

The detection of chromosomal rearrangements in childhood acute lymphoid leukemia (ALL) and acute myeloid leukemia (AML) plays an important role for the identification of clinical relevant subtypes. The cytogenetic definition of karyotype abnormalities led to the molecular genetic characterization of many translocation breakpoints and the identification of the involved genes. The expression of the fusion transcripts associated with these rearrangements provides the basis for the refinement and up-scaling of screening technologies. Recently, a multiplex-PCR strategy has been reported that allows the simultaneous detection of 28 different chromosomal aberrations that generate over 80 different fusion transcripts (Pallisgaard et al., 1998). We have used this multiplex-assay (HemaVision™, DNA Technology) for the analysis of bone marrow and peripheral blood samples of AML patients registered in the Austrian AML-BFM 93 and 98 studies. So far, we have studied 15 cases. In eleven we detected a specific fusion transcript, whereas four samples were negative for all 28 translocation products. Except for one case with a MLL/AF6 fusion transcript indicating a t(6;11), all results concurred with the cytogenetic findings. In this patient, the specific translocation was missed, although an 11q23 abnormality was obvious. In addition, we have successfully analyzed methanol/acetic acid fixed cells that had been used for cytogenetic analysis and stored for up to six years at -20C. The possibility to use such fixed material as an additional RNA source facilitates retrospective studies and analysis of cases with restricted amounts of material. The preliminary results of our analyses show that this multiplex RT-PCR test is advantageous to other screening methods, because it covers a broad spectrum of genetic alterations. It may yield new information on their frequencies and provide the information necessary for minimal residual disease studies. Moreover, it will be useful for refinement of leukemia diagnosis and may become relevant for the development of a more individualized therapeutic approach.

Program Nr: 1827 from the 1999 ASHG Annual Meeting

Molecular genetic basis for attenuated familial adenomatous polyposis. *L.-K. Su[1], C.J. Barnes[2], W. Yao[1], Y. Qi[1], P.M. Lynch[2], G. Steinbach[2]*. 1) Cancer Biology, M. D. Anderson Cancer Center, Houston, TX; 2) Gastrointestinal Oncology and Digestive Diseases, M. D. Anderson Cancer Center, Houston, TX.

Familial adenomatous polyposis (FAP) is an autosomal dominant inherited disease that predisposes patients to colorectal cancer. FAP is caused by germline mutations of the *APC* gene. Most FAP patients develop hundreds to thousands of benign colorectal tumors starting in their second decade of life. Patients with attenuated FAP (AFAP) develop fewer tumors and at older age than those with typical FAP. It is clear that patients carrying germline mutations in three specific regions of *APC* usually develop AFAP; these are the first 4 coding exons, the alternatively spliced region of exon 9 (ASRE9), and the 3' half of the coding region. However, it is not clear why mutations in these regions of *APC* cause AFAP.

We investigated the mechanism for AFAP in patients carrying a germline mutation in the ASRE9 of *APC*. We found that transfection of the *APC* cDNA without ASRE9 downregulated catenin mediated transcription as efficiently as transfection of the wild *APC* cDNA did. We then examined somatic mutations in exon 15 of *APC* in 9 tumors from two patients carrying this germline mutation. Every tumor was found to have somatic mutation and 5 of them had mutations in both alleles. A specific mutation was found in the germline mutant allele in 4 of these tumors; although it had rarely been found before in sporadic or FAP associated colorectal tumors.

Our results show that somatic mutations of both *APC* alleles are required for the formation of most colorectal tumors in these patients, therefore fewer tumors are developed in them. However, these patients are still predisposed to colorectal tumorigenesis because some mutations that occur frequently and do not completely inactivate the wild type *APC* allele can inactivate alleles of *APC* carrying germline mutations in ASER9.

Program Nr: 1828 from the 1999 ASHG Annual Meeting

CDKN2A mutations in Canadian malignant melanoma families. *A.M. Summers[1,2], D. Hogg[3], M. Shennan[2], N. Lassam[2,3].* 1) Genetics Program, North York General Hosp, North York, ON, Canada; 2) Toronto Sunnybrook Regional Cancer Centre, Toronto, Canada; 3) Department of Medicine, University of Toronto, Toronto, Canada.

Cutaneous malignant melanoma (CMM) is a serious but potentially preventable skin cancer with a lifetime risk for Canadians of 1/108. About 10% of CMM is inherited in an autosomal dominant fashion with variable penetrance. We have argued that individuals with a genetic predisposition to melanoma represent a high-risk population that should benefit from surveillance programmes. To test this hypothesis, we established a familial melanoma clinic at the Toronto Sunnybrook Regional Cancer Centre in 1996. From the outset, we have used PCR-sequencing combined with a yeast two-hybrid system to identify functionally significant mutations in CDKN2A. To date we have enrolled 155 families in the clinic and have sequenced CDKN2A in both affected and unaffected members of 105 kindreds. Among all 105 families, we have observed 33 germline mutations in CDKN2A (31.4%) including five mutations in the noncoding region at position -34. Among the 37 families with three or more affected members (AFM), the rate of germline mutations increases to 21/37 (56.8%). Thus, it is likely that many of our families with only two affected members merely represent a chance association. Our preliminary data currently indicate that the factors most predictive of finding a germline CDKN2A mutation in a melanoma-prone family include: 1) three or more AFM; 2) one or more cases of pancreatic cancer, 3) multiple primary melanomas; and 4) age of onset 30 years or under, in order of decreasing importance. Although we are steadily accruing families, it is too early to comment on issues such as penetrance, genotype/phenotype correlation, or interactions between CDKN2A and other genes or environmental factors. In the meantime, we can provide persons at risk for CMM with intensive skin surveillance both through self-examination and regular examination by a dermatologist.

Program Nr: 1829 from the 1999 ASHG Annual Meeting

A ROLE OF MEN1 IN TRANSCRIPTIONAL REGULATION AND TELOMERE FUNCTION. *K. Suphapeetiporn, D. Walpita, T. Ashley, A. Bale.* Genetics, Yale University, New Haven, CT.

MEN1 is an autosomal dominant familial cancer syndrome characterized by pituitary, parathyroid, pancreatic islet and carcinoid tumors. The MEN1 gene encodes a nuclear protein of unknown function. Biochemical studies suggest that the gene may regulate the JUN family, and in particular is a negative regulator of JUND (Agarwal et al., 1999). To investigate the function of MEN1, we constructed stable, tet-repressible carcinoid cell lines. Induction of MEN1 expression inhibited colony formation and thymidine incorporation. The Drosophila homolog of JUN induces expression of dpp, the TGF beta homolog. As predicted by the Drosophila model, TGF beta was induced in the absence of MEN1 and strongly repressed by switching on MEN1 in carcinoid cells. MEN1 is highly expressed in testes, suggesting the possibility of a role in meiosis and in recombination and repair. We determined the localization of MEN1 protein on meiotic chromosomes by immunostaining mouse spermatocytes with an antibody raised against an MEN1 peptide identical in human and mouse. The MEN1 protein localized specifically to telomeres during all stages of prophase. Many cancer predisposition genes (e.g., ATM, BRCA1 and 2) have specific localizations on meiotic chromosomes which relate to their function in mitosis and DNA repair. The localization of MEN1 on meiotic chromosomes suggests the possibility of a role in telomere regulation, which appears to be aberrant in all human tumors.

Program Nr: 1830 from the 1999 ASHG Annual Meeting

Ovarian and breast cancer risks to women in families with 2 or more cases of ovarian cancer. *S. Sutcliffe[1], P. Pharoah[1], D. Easton[2], B. Ponder[1]*. 1) Dept of Oncology, Strangeways Research Labs, Cambridge, U.K; 2) CRC Genetic Epidemiology Unit, Strangeways Research Labs, Cambridge,U.K.

There are few published estimates of the risk of developing breast or ovarian cancer in women with a strong family history of ovarian cancer. These women are typical of those presenting to family cancer clinics for counseling and management, so accurate risk estimates for both breast and ovarian cancer are needed. We have estimated these risks in women from families with two or more confirmed ovarian cancers in first degree relatives using data from the UKCCCR Familial Ovarian Cancer Register. A total of 10757 person years of follow-up data from 309 families were used for the ovarian cancer risk analysis and 11804 person years of follow-up data from 314 families for the breast cancer risk analysis. The number of observed cancers was compared with the number expected based on national, age, sex and period-specific incidence rates for England and Wales. The relative risk of ovarian cancer was found to be 6.70 (95% CI 3.46 - 11.70) and that of breast cancer 2.23 (1.48 - 3.22). When restricting the analysis to families that had tested negative for mutations in the breast/ovarian cancer susceptibility genes, BRCA1 and BRCA2, the ovarian cancer risk was 11.31 (3.64 - 26.39) and that of breast cancer 2.68 (1.22 - 5.09). In addition to its clinical importance, this finding suggests that in these families other breast/ovarian cancer genes are segregating or that BRCA1/2 mutations have been missed. More detailed subgroup analyses will also be presented.

Program Nr: 1831 from the 1999 ASHG Annual Meeting

Inherited Breast/Ovarian Cancer in African-American Families. *R. Sutphen[1, 2], T.M. Ferlita[1]*. 1) H. Lee Moffitt Cancer Center, Lifetime Ca Screening, Tampa, FL; 2) Dept. Clinical Genetics #7815, All Children's Hosp., St. Petersburg, FL.

The majority of studies of BRCA1 and BRCA2 have been performed in Caucasian families with a strong family history of breast cancer and few of the studies have included African-Americans. A few, novel recurrent mutations have been identified in limited studies, indicating that founder mutations are present in the African-American population and additional studies are needed. Related data regarding risk perception and attitudes about genetic testing among African-Americans is also limited. We have identified 43 living patients from the Moffitt Cancer Center Registry with 1)breast cancer diagnosed at age 45 or younger and/or 2)a family history of breast and/or ovarian cancer. Genetic counseling and testing are being offered to these women, including complete sequencing of the coding region of BRCA1 and BRCA2. Psychological measures are employed to explore risk perception, cancer beliefs and attitudes, and distress. Thus far, one African-American woman who was diagnosed with breast cancer at age 29 and who has no known family history of breast or ovarian cancer has had gene testing. A novel BRCA1 mutation, 926ins10, was detected. The preliminary results of our study will be presented, including results of laboratory testing and psychological assessment.

Program Nr: 1832 from the 1999 ASHG Annual Meeting

BRCA1 Mutations: Haplotype and Phenotype Analysis Revisited. *C.I. Szabo[1], E. Fleischmann[2], S. Neuhausen[3], P. Devilee[4], D. Goldgar[1], T. Wagner[2], and the Breast Cancer Linkage Consortium[5]*. 1) Unit of Genetic Epidemiology, International Agency for Research on Cancer, Lyon, France; 2) Department of OB&GYN, Univ. Klinik fur Frauenheilkunde, Vienna, Austria; 3) Genetic Epidemiology, University of Utah, Salt Lake City, UT; 4) Dept. of Human & Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands; 5) A full listing of contributors to the study will be available in the presentation.

Identification of BRCA1 and BRCA2 and subsequent characterization of mutations in these genes revealed that while the majority of mutations are unique, several are found in multiple families. These frequently described mutations appear to be population-specific (2804delAA in the Netherlands) or, in some instances (5382insC), occur in geographically and ethnically diverse families. Initial international collaborative studies defined the haplotype associated with six frequently reported BRCA1 and nine BRCA2 mutations, and explored mutation-specific phenotypic variation. Two BRCA1 mutations showed evidence for multiple independent mutation origins, with the remainder resulting from ancestral founder events arising between 9 and >100 generations ago. In some instances, mutation-specific differences in phenotype (tumor site) and age of onset were observed. Data for BRCA1, obtained from a larger (5X) series of 185delAG and 5382insC mutation carriers representing greater geographic and ethnic diversity will be presented. Haplotypes associated with previously uncharacterized geographically diverse (300T>G) and population-restricted (1806C>T, 3600del11) BRCA1 mutations will also be described. Additional markers are being incorporated for finer resolution mapping of haplotypes over a longer distances. Age of each mutation will be estimated, and a phylogenetic tree of haplotype alterations will be derived for correlation with ethnic and geographic information. In this manner the place of origin and subsequent spread of these mutations to other regions of Europe, and the relevant populations in the United States, Canada and Australia will be characterized. Preliminary analyses of 185delAG and 5382insC indicate extensive haplotype sharing of an ~900 kb region.

Program Nr: 1833 from the 1999 ASHG Annual Meeting

The effects of glutathione-related genes and cigarette smoking on lung cancer short-term survival. *K. Taniguchi, P. Yang, R. Marks, T. Lesnick, A. Yokomizo, J. Sloan, D. Miller, J. Jett, H. Tazelaar, E. Edell, D. Smith, W. Liu.* Mayo Clinic, Rochester, MN.

Genes encoding glutathione (GSH) synthetase and transferases could be useful in predicting lung cancer prognosis because several GSH-related metabolic enzymes could inactivate anti-cancer drugs. We hypothesize that patients with genotypes corresponding to low activity levels of GSH-related enzymes are associated with better lung cancer survival. In a pilot study, we included 313 patients diagnosed in our institution and followed up for one year. Each patient completed a baseline interview regarding cigarette smoking status and family history of cancer and other pulmonary diseases. Medical records were abstracted to obtain clinical staging, histopathology, and treatment data. One hundred and six patients were genotyped at four GSH-related loci: *GSTP1, GSTM1, GSTT1,* and g-*GCS*. The cutpoint of 40 pack-years was used for defining light/moderate vs heavy smokers in our analysis. There were 194 males (62%) and 45 never-smokers (14%). The crude one-year survival rate was 51%. For cases with early- vs late-stage at diagnosis, the survival rates were 83% and 42%, respectively. The survival rates for patients with *null* genotype at two of the four loci (*NN*) was compared to patients with a *positive* genotype at one or both loci (*other*). We found an overall higher survival rate for patients with *NN* (73-86%) than patients with *other* genotypes (59-60%). In heavy smokers, the one-year survival rate was 71-100% in NN group compared to the 65-70% rate otherwise. This disparity between contrasting genotypes among late-stage patients (who usually underwent chemotherapy) was larger than among early-stage patients (who were mainly treated by surgical resection). The survival rate among late-stage patients with NN was approximately 20% higher than patients with *other* genotype. Among late-stage patients who were heavy smokers, the survival rate was 43-67% for NN and 17-33% for *other* genotype. These preliminary data reflect a clinically significant effect although questions involving confounding influences remain. Further investigation with a larger sample is warranted.

Program Nr: 1834 from the 1999 ASHG Annual Meeting

Novel genes in Acute Myeloid Leukemia with Trisomy 8. *S.M. Tanner[1], K. Virtaneva[1], L. Rush[1], M.A. Caligiuri[2], C.D. Bloomfield[2], K. Mrózek[2], C. Plass[1], R. Krahe[1], A. de la Chapelle[1]*. 1) Human Cancer Genetics, Ohio State University, Columbus OH; 2) Comprehensive Cancer Center, Ohio State University, Columbus OH.

Acute myeloid leukemia (AML) is a histopathologically, cytogenetically, and clinically heterogeneous neoplasia. About 55% of de novo AML cases reveal clonal cytogenetic abnormalities while the remaining ones have normal karyotypes. Trisomy 8 is the most frequently encountered aneuploidy in AML (AML+8), either as a sole abnormality or as part of a more complex pattern of abnormalities. AML+8 is associated with poor prognosis, and the molecular mechanisms related to the role of trisomy 8 in AML are unknown. We entertain two hypotheses. First, in concordance with recent findings in trisomy 11 AML, molecular rearrangements of (a) gene(s) on chromosome 8 might be associated with AML+8. Second, trisomy 8 might cause changes in gene expression patterns that lead to or contribute to AML. In order to address both possibilities, we performed cDNA based Representational Difference Analysis (RDA) to compare a pool of AML+8 cells with a pool of AML cells with normal karyotypes. To exclude cryptic chromosome rearrangements, the cytogenetic findings were confirmed by spectral karyotyping (SKY). Using the AML+8 cells as the RDA-tester, we prepared and sequenced 209 different clones from mRNAs presumably present in trisomic and absent in disomic cells. A subsequent BLAST search of GenBank and TIGR databases identified 27 different known genes, five ESTs, and five unknown sequences. We selected two genes, two ESTs, and one unknown sequence for detailed studies and confirmed by comparative RT-PCR that the two genes and one EST were indeed more highly expressed in trisomic than in disomic AML cells. This EST was shown to be expressed only in brain and AML+8 cells, and its genomic location is currently being defined. The second EST was mapped to chromosome 5p13 and the unknown sequence was located on chromosome 14q32. The cDNA sequences and the genomic structure of these candidate leukemia genes are under investigation. Gene expression studies and mutation analysis will help to elucidate the role of these novel genes in the pathogenesis of AML+8.

Program Nr: 1835 from the 1999 ASHG Annual Meeting

YAC-BAC contig of the Carney complex (CNC) critical region on 2p16 and copy number gain of 2p16 in CNC tumors: evidence for a novel oncogene? *S.E. Taymans[1], L.S. Kirschner[1], S. Pack[2], C. Giatzakis[1], C.A. Stratakis[1]*. 1) Genetics & Endocrinology/DEB/NICHD/NIH, Bethesda, MD; 2) Laboratory of Pathology/NCI/NIH, Bethesda, MD.

Carney complex (CNC)(MIM 160980) is an autosomal dominant multiple endocrine neoplasia and lentiginosis syndrome characterized by spotty skin pigmentation, cardiac, skin, and breast myxomas, and endocrine tumors. Linkage analysis placed the first CNC locus at 2p15-p16, between D2S391-D2S378, but a second locus was recently identified at 17q22-24. We are using both positional cloning techniques and molecular cytogenetic studies of tumors to identify the gene causing CNC. A high resolution physical map of the CNC locus on 2p16 was built, using YAC and BAC clones, by STS and EST content mapping and chromosomal walking. Over 100 ESTs from online databases of 2p15-p16 were screened against our contig. 78 unique ESTs were placed in the contig, and 30 were excluded from the region. Four new genes were identified, fully characterized, and excluded as candidates. Positional cloning of genes for familial tumor syndromes is assisted by use of tumor tissue from affected patients. We used polymorphic STSs and BAC clones from the CNC locus on 2p16 to examine DNA from various CNC tumors or tumor cell lines for alterations. Loss of heterozygosity was not present for STSs from 2p16 in CNC tumors. In normal tissue the BACs hybridized specifically to 2p15-p16 as the expected 2 copies. However, in CNC tumors 2p15-p16 BACs were amplified. The degree of amplification varied, and not all BACs were amplified in every tumor. A particular BAC clone from the core of the CNC critical region, containing 5 unique ESTs, including CNC candidate genes, showed the most consistent and greatest level of amplification. This BAC was amplified several hundred fold in pituitary and thyroid tumors; but was specifically deleted in 2/8 adrenocortical tumors, suggesting that deletion of one allele precedes amplification of the other. 2p16 participates in tumor formation in CNC with apparent gain of its genomic material in the process. Together, our contig and cytogenetic data provide an excellent resource for identifying the CNC gene.

Program Nr: 1836 from the 1999 ASHG Annual Meeting

FISH studies of peripheral blood cells for monitoring therapy in chronic myeloid leukemia (CML). *A. Tchirkov, G. Briançon, M. Giollant, P. Travade, G. Lacroute, F. Ait-Ouaret, F.A. Shah, P. Malet.* University of Auvergne, Faculty of Medicine, Clermont-Ferrand, France.

Conventional cytogenetic studies are useful to monitor therapy in CML patients, but regular follow-up requires frequent invasive bone marrow (BM) aspirates. In this study we evaluated the quantification of CML cells using FISH for BCR-ABL in peripheral blood (PB) samples, which can be easier collected from the patients, as a method of monitoring the response to interferon-alpha therapy. The metaphase and/or interphase FISH investigations were performed on cultured and uncultured PB, and the results were compared with BM cytogenetics. In PB cultures, leukemic cells were efficiently detected in 15 samples obtained from 10 patients when there was no hematologic response. In contrast, the percentage of BCR-ABL-positive cells was non significant in 19 samples obtained from 15 patients during complete hematological remission. However, these patients had substantial numbers of Philadelphia-positive cells in BM. This suggests that normal BCR-ABL-negative PB cells may be selected by culture conditions and the FISH approach with PB cultures is not adequate for evaluating residual disease in CML patients with hematologic response. Importantly, the levels of leukemic cells detected in uncultured PB specimens from these patients processed directly for FISH studies compared favourably with BM data. Thus, interphase FISH analysis of uncultured PB may be useful for follow-up investigations in CML patients.

Program Nr: 1837 from the 1999 ASHG Annual Meeting

Geneological and genetic correlation in Finnish Multiple Endocrine Neoplasia Type 1 (MEN 1) families. *B.T. Teh[1], S. Kytölä[1], F.K. Wong[1], A. Villablanca[1], B. Nord[1], C. Larsson[1], T. Ebeling[2], L. Ukkola[2], O. Vierimaa[2], P. Salmela[2], J. Leisti[2]*. 1) Molecular Medicine, Karolinska Hospital, Stockholm, Sweden; 2) Clinical Genetics and Internal Medicine, Oulu University Hospital, Oulu, Finland.

In Finland more than 30 monogenic disorders are known to be clustered in specific regions. The occurrence of MEN 1 shows geographical concentration, with the majority of hitherto identified Finnish families originating from and residing in the Northern parts of the country. In this study we attempted to characterize the spectrum of MEN1 mutations in the Finnish population and to correlate the mutations with our geneological data. Twenty-six clinically confirmed MEN 1 cases representing twenty two families and four isolated MEN 1 cases were screened for MEN1 mutations by SSCA and DNA sequencing. In addition, the disease-associated haplotypes of each case was determined by genotyping 11q13 markers spanning the MEN1 region. Six different mutations were identified in 17 of the cases, while in the remaining nine cases no mutation has been identified yet. 1466del12 was seen in nine families, 1657insC in three families, R527X in two families, D418N,G42A, and 359del4 were found in one family each. All nine families with the 1466del12 mutation were shown to share the same affected haplotypes, thus indicating that the mutation occurred on a common ancestral chromosome. This was confirmed by subsequent geneological search which traced the roots of eight families to a small village approximately 45 kilometers East of Oulu, where the founder couple was born in 1705 and 1709, respectively. In the ninth family the geneological connection has not yet been established. The pedigree consists of about 6-10 generations, when counted from the present-day patients. Three families with 1657insC-mutation could be traced back to a couple living 200 kilometres Northeast from Oulu born in 1844 and 1846, who were four generations from the youngest living patients. In conclusion, the genetic-genealogical correlation established two founder MEN1 mutations in Northern Finland. It is therefore worthwhile to screen for these two mutation in new Finnish MEN1 patients.

Program Nr: 1838 from the 1999 ASHG Annual Meeting

ATM Mutations in Breast Cancer Patients with Early-onset or Family History. *S.N. Teraoka[1], K.E. Malone[2], E.A. Ostrander[3], J.R. Daling[2], P. Concannon[1].* 1) The Program in Molecular Genetics, Virginia Mason Research Ctr, Seattle, WA; 2) Division of Public Health, Fred Hutchinson Cancer Research Center, Seattle, WA; 3) Division of Clinical Research, Fred Hutchinson Cancer Research Center, Seattle, WA.

We are screening for mutations in the ATM gene among individuals drawn from a population-based study of breast cancer in a 3 county area of Western Washington. Breast cancer cases have been selected based on meeting one of 2 criteria, early-onset (diagnosed by age 35), or having a first degree relative with breast cancer. To date, we have screened 142 cases and 80 matched controls. The 62 ATM coding exons were amplified by multiplex PCR and screened by SSCP. No truncation or deletion mutations were detected. However, possible ATM missense mutations were detected in 7.7% (11/142) of total cases, versus 1.25% (1/80) in controls. All 11 of the cases with possible mutations had some family history of breast cancer. Of 64 cases with a first degree relative with breast cancer, 8 (12.5%) had ATM missense mutations. Among the 99 cases diagnosed at age 35 or less, 4 (4%) had ATM missense mutations. An additional 27 individuals with rare variants that resulted in amino acid substitutions were also identified (17 in cases, 10 in controls). Some of these rare variants may also represent mutations, but their effects will require further functional studies. We have recently shifted our screening approach to utilize denaturing HPLC and have obtained increased sensitivity over SSCP for the detection of single nucleotide substitution in the ATM gene.

Program Nr: 1839 from the 1999 ASHG Annual Meeting

Rearrangements of both chromosomes 22 in a rhabdoid tumor. *I. Teshima, G. Nie, P.S. Thorner.* Pediatric Lab Medicine, Hosp Sick Children, Toronto, ON, Canada.

Malignant rhabdoid tumors are aggressive tumors that usually occur in children less than two years of age. The tumor was originally described in kidney but can occur in brain, liver, and soft tissues. Classification has been difficult because histological characteristics are variable depending on location. The cell of origin is not known. Because the prognosis for rhabdoid tumors is poor, aggressive therapy is required. Accurate diagnosis is thus important and now includes genetic criteria. Cytogenetic studies of rhabdoid tumors had suggested a common genetic basis for the brain, renal and extrarenal rhabdoid tumors with monosomy 22 being the most common finding. The region involved was localized to 22q11.2. A tumor suppressor gene was postulated with homozygous alterations required for oncogenesis. The candidate gene, INI1, encoding a member of the chromatin remodeling SWI/SNF multiprotein complex, has now been identified. The SWI/SNF complex is capable of opening up nucleosomes to facilitate transcription. Biegel et al.(Cancer Res 59:74-9; 1999) in a study of rhabdoid tumors found homozyous alterations of INI1 in 15 tumors and mutations in 14 tumors; germline mutations were found in 4 of the children with these tumors. An extrarenal rhabdoid tumor presented as a soft tissue tumor in a 9-year-old boy. WT1 was strongly expressed. G-banding of tumor cells revealed two somatic rearrangements, each involving a different homologue of chromosome 22 with breakpoints 22q11.2~12. A translocation between 15 and 22 and a complex rearrangement involving 1, 6, 13, and 22 were present along with a normal 46,XY cell line. This was demonstrated using FISH probes for TUPLE1 (22q11.2)/ARSA (22q13) and BCR (22q11.2), SKY, and paints for 1, 6, 13 and 22. At least 14 cases of rhabdoid tumors with one translocation involving chromosome 22 have been reported. While the loss of the INI1 gene was not directly shown in this case, the presence of two rearrangements in the vicinity of this gene is consistent with the requirement for inactivation of both alleles in the development of this tumor and served as a diagnostic aid for the identification of the tumor in this case.

Program Nr: 1840 from the 1999 ASHG Annual Meeting

Detection of RB1 deletion by primed in situ labeling (PRINS). *S.A. Tharapel[1], J.S. Kadandale[2].* 1) Dept Pediatrics/Div Genetics, VA Med Ctr and Univ Tennessee, Memphis, TN; 2) Clinical and Molecular Cytogenetics Laboratory, Department of Pediatrics, University of Tennessee, Memphis, TN.

Chromosome band 13q14 which contains the retinoblastoma gene (RB1), has been implicated in an array of human malignancies including retinal tumors and certain leukemias. Approximately 10% of B-cell chronic lymphocytic leukemias (CLL) are known to have structural abnormality involving the 13q14 region. Commercial FISH probes have enhanced our ability to identify small deletions including RB1. To assess the feasibility and to develop a less expensive alternative to FISH, we modified the primed in situ labeling (PRINS) technique to enable detection of RB1 deletion. Our study group consisted of 5 patients with 13q14 deletion (demonstrated by convetional cytogenetics and FISH), and 5 normal controls. The method involved use of locus-specific oligonucleotide primers for the RB1 locus in the presence of dATP, dCTP, dGTP, dTTP and digoxigenen-11-dUTP, Tris-HCl, KCl, MgCl2, BSA and Taq DNA polymerase. Annealing and extension were performed on one-day-old chromosome preparations. For each patient and control, 30 cells were analyzed under a fluorescence microscope. The patients' cells were remarkable for absence of labeling for RB1 on one chromosome 13, consistent with the deletion. A normal pattern was seen in the 5 controls. In view of our experience with PRINS localizing of other genes and regions (SRY, SOX3, GABRB3, SNRPN, D22S1638, D22S1648 and AZF) (our unpublished data) we conclude that PRINS is a sensitive and valuable alternative to FISH for the detection of constitutional and acquired chromosomal deletions and rare translocations.

Program Nr: 1841 from the 1999 ASHG Annual Meeting

Evidence for Genotype-Phenotype Correlations in BRCA2. *D.J Thompson, D.F Easton, on behalf of the Breast Cancer Linkage Consortium.* CRC Genetic Epidemiology Unit, University of Cambridge, Cambridge, ENGLAND.

Mutations in BRCA2 confer susceptibility to breast and ovarian cancer. In 1997 Gayther *et al.* identified a 3.3kb region of exon 11 in which mutations appeared to confer a higher risk of ovarian cancer relative to breast cancer than mutations elsewhere in the gene. This section was named the Ovarian Cancer Cluster Region (OCCR). The OCCR coincides with the coding region for a sequence of internal repeats in the BRCA2 protein which have been shown to interact with the RAD51 DNA repair gene and are thought to play an important role in BRCA2's function.

Here we present the results of a study based on a much larger set of 164 families with BRCA2 mutations (including 69 with at least one case of ovarian cancer) to test and evaluate the OCCR effect. Sixty-seven of these families had mutations in the OCCR.

The odds ratio for ovarian versus breast cancer in families with mutations in the OCCR, relative to non-OCCR mutations, was significantly greater than one (odds ratio=3.86, P<.0001, using a randomisation test), confirming the observation that cancer risks are different in this part of the gene. No other significant differences in risk by mutation position were observed.

This does not differentiate between an increased ovarian risk, a reduced breast cancer risk for OCCR mutations relative to the rest of the gene, or a combination of both. To establish the size and significance of each effect the cancer risks for OCCR mutations relative to non-OCCR mutations were estimated. Population frequencies of each BRCA2 mutation are unknown, so risks were estimated using a likelihood conditional on the set of mutations observed in the study.

The maximum likelihood estimates of the relative risks were 0.65 for breast cancer (95% CI=0.48-0.88) and 1.34 for ovarian cancer (95% CI=0.86-2.09). Thus there is significant evidence that the risk of breast cancer is lower for mutations within the OCCR than for mutations outside it, but the evidence that OCCR mutations confer a higher risk of ovarian cancer is weaker.

Program Nr: 1842 from the 1999 ASHG Annual Meeting

Homozygous deletion of CDKN2A (p16/p14) in neuroblastomas. *P.M. Thompson[1], J.M. Maris[1], M.D. Hogarty[2], R.C. Seeger[2], C.P. Reynolds[2], G.M. Brodeur[1], P.S. White[1].* 1) Division of Oncology, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Department of Pediatrics, University of Southern California School of Medicine, Los Angeles, CA.

Neuroblastoma (NB) is the most common pediatric extra-cranial solid tumor. Loss of heterozygosity of several specific genomic regions is frequently observed in NB tumors and cell lines, but homozygous deletion (HD) has been reported in only two cell lines, once each at the NF1 and CDKN2A tumor suppressor genes (TSG). Furthermore, no TSG mutations have been reproducibly identified in NB. We performed a comprehensive search for homozygous deletions (HD), indicative of a disrupted TSG, in a panel of 46 NB cell lines. Our initial search focused upon a well-characterized consensus region of hemizygous deletion (SRO) at 1p36.3 which occurs in 35% of NB tumors. We used a PCR assay to screen each cell with 163 1p36 markers, yielding resolutions of 20 kb within the 1p36.3 SRO and 250 kb throughout 1p36. No homozygous deletions were detected. This approach was expanded to survey 21 known TSGs, specifically targeting intragenic regions most frequently disrupted in other malignancies. HD was detected only at the CDKN2A gene at 9p21 and was observed in 4 of 46 cell lines. The CDKN2A gene produces two overlapping proteins, p16 and p14, which are involved in cell cycle regulation and are disrupted in many malignancies. The observed region of HD included all exons of both CDKN2A and the neighboring CDKN2B (p15) locus for cell line L-AN-6; exons 1 and 2 of CDKN2A and all of CDKN2B for CHLA-174; exons 1 and 2 of CDKN2A for CHLA-179, and only 104 base pairs of exon 2 for CHLA-101. All four rearrangements are predicted to completely or extensively disrupt the coding region of both p16 and p14. HD was observed in a corresponding primary tumor sample only for CHLA-174, which is the first reported incidence of HD in a NB primary tumor, but was not present in constitutional samples. These results suggest that large HDs do not occur within 1p36 in NB, that most known TSGs are not homozygously deleted, and that biallelic inactivation of CDKN2A may be a significant contributor to tumorigenicity in NB.

Program Nr: 1843 from the 1999 ASHG Annual Meeting

Varied effects of a BRCA2 founder mutation. *S. Thorlacius[1], G.H. Olafsdottir[2], L. Tryggvadottir[2], H. Tulinius[2], J.E. Eyfjord[1]*. 1) Dept Molecular & Cell Biol, Icelandic Cancer Society, Reykjavik, Iceland; 2) Cancer Registry, Icelandic Cancer Society, Reykjavik, Iceland.

Breast carcinoma is the most common malignancy among women in developed countries. The contribution of the BRCA1 and BRCA2 genes has been estimated to be 5-10% of all breast cancer. Based on breast cancer families with multiple and/or early-onset cases, estimates of the lifetime risk of breast cancer in carriers of BRCA1 or BRCA2 mutations is varied but may be as high as 85%. Knowledge on the interaction between environmental agents and BRCA mutations in mutation carriers remains unclear. The Icelandic population has a strong founder BRCA2 mutation. We have detected the BRCA2 999del5 germline mutation in 0.6% of the population, 8% of female breast cancer patients, and in 40% of males with breast cancer. The mutation is strongly associated with younger onset of female breast cancer. In a population based study we estimated the risk of breast cancer for a 999del5 mutation carrier to be 37% at age 70. We have studied various BRCA2 families and find different pattern of disease. In some families male mutation carriers develop prostate cancer while in others they are at increased risk of breast cancer. These two cancer types are rarely found occurring in the same families. There are indications of anticipation in BRCA2 families, mainly seen as a higher penetrance or earlier onset in younger generations. It is clear from our studies that factors other than BRCA2 influence cancer risk in this population. These findings suggest that individual risk assessment will always have to take account of family history.

Program Nr: 1844 from the 1999 ASHG Annual Meeting

HER2/neu analysis of paraffin-embedded thymoma and thymic carcinoma specimens. *V.C. Thurston[1], P. Loehrer[2], J. Henley[3], G.H. Vance[1]*. 1) Dept Medical & Molecular Gen, Indiana Univ, Indianapolis, IN; 2) Dept of Medicine, Indiana Univ, Indianapolis, IN; 3) Dept of Pathology, Indiana Univ, Indianapolis, IN.

Thymomas and thymic carcinomas are malignant neoplasms derived from thymic epithelial cells and together represent the most common primary anterior mediastinal tumor in adults. The majority of thymic neoplasms are cytologically benign, encapsulated tumors known as thymomas. Despite their benign appearance, approximately 30-40% of thymomas exhibit malignant behavior and progress or recur with locally advanced, unresectable disease or distant metastases. There exists a lack of reliable pathological or molecular correlates of tumor aggressiveness. We present preliminary data of FISH analysis (Oncor INFORM "HER2/neu" probes) on nine of twenty-five paraffin-embedded tumors. Pathologic diagnosis of the specimens included two thymic carcinomas and seven thymomas (two encapsulated, three invasive and two metastatic thymomas). Forty nuclei were scored per specimen. An average of 1.83 signals/nuclei was found in all samples with an average of 1.9 signals/nuclei in thymic carcinomas; 1.79 signals/nuclei in invasive thymomas; 1.91 signals/nuclei in metastatic thymomas and 1.86 signals/nuclei in thymomas. These results are considered normal. Thus, in this initial group of tumors there is no evidence of HER2/neu amplification.

Program Nr: 1845 from the 1999 ASHG Annual Meeting

Contribution of founder BRCA1 and BRCA2 mutations in French Canadian HBC and HBOC families. *P.N. Tonin[1], D. Provencher[2,3], A.-M. Mes-Masson[2,3], S. Narod[4], W. Foulkes[1], P. Ghadirian[3,5]*. 1) McGill U & MUHC; 2) CHUM Hopital Notre Dame; 3) U of Montreal; 4) Women's College Hospital; 5) CHUM Hopital Hotel Dieu.

Germ-line BRCA1 and BRCA2 mutations account for a large proportion of hereditary breast cancer (HBC) and breast-ovarian cancer (HBOC) families. Mutation analysis has been facilitated by the identification of founder mutations in well defined ethnic groups. Recently we reported founder BRCA1 and BRCA2 mutations in 40/97 families of French Canadian ancestry containing a minimum of three cases of breast and/or ovarian cancer. Here we examine the contribution of the founder mutations in a large series of French Canadian families (n=123) with a minimum of four cases of breast-(dx less than 66 yrs) and/or ovarian-cancer. Genomic DNA from an affected case from each family was analyzed for the presence of BRCA1 (C4446T, 2953del3+C and 3768insA) and BRCA2 (2816insA, G6085T, 6503delTT and 8765delAG) founder mutations. Founder BRCA1 (n=28) and BRCA2 (n=30) mutations were found in 58 families. The BRCA1 C4446T (n=23) and BRCA2 8765delAG (n=15) mutations were the most common mutations identified. Families which harbour the founder mutations were not restricted to Montreal area of ascertainment. A founder BRCA1 mutation was more often identifed in a HBOC (20/54) than a HBC (8/69) family; whereas, a founder BRCA2 mutation was more often identified in a HBC (23/69) than a HBOC (7/54) family. Mutations were identified in all four families (BRCA1 (n=1); BRCA2 (n=3)) with at least one male breast cancer case. The identification of founder mutations will facilitate carrier detection and genetic counselling in French Canadian HBC and HBOC families. In our continuing examinination of founder mutation-negative families, we have uncovered three mutations not previously described in families of French Canadian descent. To date, the analysis of mutation-negative families has revealed that these new mutations are not common in this population. The sequence analysis of BRCA1 and/or BRCA2 in index cases from of a subset of the mutation-negative families (n=10) support the hypothesis that additional factors may be contributing to HBC and HBOC families in the French Canadian population.

Program Nr: 1846 from the 1999 ASHG Annual Meeting

Gene variation and cancer risk: generation of complex haplotypes at the *BLM, WRN* and *ERCC1* loci. *D. Trikka, Z. Fang, D.L. Nelson.* Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

The aim of the project is to develop biallelic markers for each of 25 genes known to be involved in carcinogenesis. Haplotypes based on these markers should provide sufficient information to determine whether certain gene variants (marked by haplotypes) can be associated with a higher or lower risk of tumor development in the general population. Using the genomic sequence data developed in our laboratory, as well as that already available in the databases, we have begun to identify 8-12 single nucleotide polymorphisms (SNPs) per gene. Steps towards the identification of SNPs include screening of BAC and PAC libraries, creation of contigs and sequence analysis of clones for each of 20 genes that have been shown to play a role in carcinogenesis. In parallel, the sequence data that is already available is used in designing PCR primers, randomly spaced along the gene, amplification of the corresponding regions and direct sequencing. The results of the sequence analysis of three genes (*BLM*, *WRN* and *ERCC1*) that were screened for SNPs in this manner are presented here. The genomic sequence of all genes was already available to us. Identification and characterization of the single base changes was carried out by PCR amplification and direct sequencing of the amplified regions in five unrelated individuals. Coding regions were avoided. Ten, fifteen and eleven SNPs were identified within the *BLM*, *WRN* and *ERCC1* genomic regions, respectively. Our data is in agreement with the expected SNP frequency of 1 every 1kb. Allele specific oligo (ASO) hybridization was subsequently used to determine the haplotypes for *BLM* and *WRN*, in 10 CEPH families and 250 samples of different ethnic background (70 Caucasians, 70 Blacks, 70 Hispanics and 40 Asians). Haplotype frequencies were ascertained by computer-assisted statistical analysis based on maximum-likelihood estimates. The haplotype data created this way will be used to compare the haplotype frequencies of human samples ascertained for specific phenotypes and thus determine the role of gene variation in the phenotypic differences selected.

Program Nr: 1847 from the 1999 ASHG Annual Meeting

Comparison of FISH & IHC Techniques in the Detection of HER-2/neu Amplification in Breast Cancers. *A.J. Tsai, M.R. Quddus, M.M. Steinhoff, U. Tantravahi.* Departments of Pathology & Molecular Cytogenetics, Women & Infants Hospital, Providence, RI.

Her-2/neu status has been found to be an important parameter in the treatment strategy of breast carcinoma. Investigators have shown that assessment of Her-2/neu status varies with technique used. The majority of the current testing for overexpression is based on immunohistochemical (IHC) staining of the gene product (protein). A fluorescent *In-situ* hybridization (FISH) gene amplification test has been introduced recently which determines amplification at the gene level. The current study was designed to compare both tests for efficient detection of the status of the oncogene in breast cancers.

Forty-six archival paraffin embedded breast cancer tissues that had previously been assessed for Her-2/neu protein amplification using IHC technique were obtained from the Department of Pathology, Women & Infants' Hospital. FISH was performed on 4m tissue sections. A DNA probe for Her-2/neu gene (Oncor) was used which directly assessed gene amplification rather than gene product. Hybridization signals were detected using Digoxigenin Rhodamine Detection Kit. A total of 50 cells were scored from each slide in a blinded fashion using a fluorescent microscope with triple band filter. Cells with >4 signals were considered to have Her-2/neu gene amplification. Signals of the adjacent normal tissue areas were counted as an internal control.

In 21.8% cases both FISH & IHC were positive for Her-2/neu amplification. In 34.4% cases FISH was positve while IHC being negative. Both were negative in 34.8% cases. Only in 8.6% cases IHC was positive and FISH being negative.

Our study revealed FISH & IHC were in agreement about Her-2/neu amplification status in 56.6% of time. The two techniques were in disagreement in approximately 43.4% of the cases, in 34.8% of cases Her-2/neu was detected by FISH & not by IHC clearly indicating that FISH is a more sensitive technique in detecting Her-2/neu gene amplification.

Program Nr: 1848 from the 1999 ASHG Annual Meeting

Identification of breast-ovarian cancer families without BRCA1 or BRCA2 mutations.. *M. Unger[1], K.L. Nathanson[1], D. Antin-Ozerkis[1], A.M. Martin[1], H.A. Shih[1], K.A. Calzone[1], T. Ward[1], S. Mazoyer[3], T. Rebbeck[2], B.L. Weber[1]*. 1) Depts of Medicine & Genetics and; 2) Dept of Biostatistics & Epidemiology, U of Penn, Phila, PA; 3) IARC, Lyon, France.

Data from the Breast Cancer Linkage Consortium suggest that 83% of families with ³4 cases of breast cancer before the age of 60 and 1 case of ovarian cancer can be linked to mutations in BRCA1 or BRCA2; or 98% if there is ³2 cases of ovarian cancer. These data have led to the hypothesis that BRCA1 and BRCA2 may fully account for all hereditary breast and ovarian cancer. Since families seen in high-risk evaluation clinics tend to have less extensive cancer histories than those studied by the BCLC, the 83% and 98% of mutations may be higher than those typically found in the population. Our objectives are: a) to determine the probability of identifying a BRCA1 and/or BRCA2 mutation in br/ov families identified through a high-risk clinic and b) to identify br/ov families without BRCA1 or BRCA2 mutations.

We selected 116 families with at least one case of female breast and ovarian cancer in the same lineage. One affected member of each family was screened for BRCA1 and BRCA2 coding region and splice junction mutations using CSGE or sequencing. To date, 67 (58%) of the 116 families carry alterations in BRCA1 and/or BRCA2. This percentage increases to 82% if the family has at least one case of breast and ovarian cancer in a single person. Six families(5%) have uncertain variants in BRCA1 or BRCA2. Testing is still pending on 10 families. Lastly, 33 families (28%) have tested negative for BRCA1 and BRCA2. In order to ensure non-coding mutations in BRCA1 are not missed by this screen, BRCA1 and BRCA2 negative families are being analyzed for large genomic deletions.

Our preliminary results suggest that incidence of BRCA1 and BRCA2 mutations in families with multiple cases of breast and ovarian cancer or a case of breast and ovarian cancer in a single individual may approach that found in the BCLC data set. However, smaller families, typically seen in a high-risk clinic may have a lower frequency of mutations.

Program Nr: 1849 from the 1999 ASHG Annual Meeting

SAGE as a tool to identify MYCN downstream pathways in Neuroblastoma. *R. Versteeg, H. Caron, P. Van Sluis, I. Roobeek, K. Boon.* Dept. of Human Genetics, Academic Medical Center, Amsterdam, Netherlands.

SAGE (Serial Analysis of Gene Expression) enables the identification and quantification of all transcripts expressed in a tissue or cell line. We have applied SAGE to identify pathways deregulated in neuroblastoma. Neuroblastoma is a childhood tumor with a heterogeneous clinical outcome. Genetic defects predictive for aggressive neuroblastoma are MYCN amplification, chromosome 1p deletions and extra copies of chromosome 17q. MYCN was identified as a transcription factor, but its downstream pathway is unknown. Analysis of chromosome 1p deletions and chromosome 17q gains revealed that the regions involved are surprisingly large and probably harbor a series of tumor suppressor genes and oncogenes with a role in neuroblastoma pathogenesis. The length of deletions and their complexity has precluded molecular identification of the involved genes so far. We have set out to apply high-througput technologies emerging from the Human Genome project to study these issues. We have established five SAGE libraries, both from MYCN amplified and from MYCN single copy neuroblastoma. We have presently sequenced 65.000 SAGE tags, each corresponding to and identifying a single transcript. By comparing four relatively small SAGE databases, we identified a series of genes potentially under control of MYCN. Northern blot analysis confirmed that 10 of them indeed are strongly induced by MYCN, thus enabling us to identify components of the MYCN downstream pathway activated in neuroblastoma. In addition, we use the database to establish gene expression profiles in neuroblastoma of the 1p35-36 and 17q regions implicated in pathogenesis. The relevant regions were defined by analysis of a panel of 200 neuroblastomas. The genes in these regions were identified on the radiation hybrid map (HGM99). Analysis of our SAGE database showed expression for about 40% of the genes in these regions. Together these data give ample new opportunities to study neuroblastoma pathogenesis and find new targets for therapeutic intervention.

Program Nr: 1850 from the 1999 ASHG Annual Meeting

Functional properties of PAX7 and PAX7-FKHR: A comparative analysis of cell response to ectopic expression of these genes. *E.V. Vorobyov[1], U. Siebers[1], K. Wilke[1], H.-G. Pauels[2], A. Julich[1], B. Dworniczak[1], J. Horst[1]*. 1) Inst. fuer Humangenetik; 2) Inst. fuer Immunologie, Westfaelische Wilhelms Univ, Muenster, Germany.

The PAX3-FKHR and PAX7-FKHR hybrid genes, resulting from similar chromosomal translocations in alveolar rhabdomyosarcoma, are assumed to play an important role in the development of this tumor. In the respective chimeric proteins C-termini of PAX3 and PAX7 are replaced by the FKHR protein moiety, that leads to functional changes of these transcriptional factors. To examine the functional properties of the intact PAX7 and chimeric PAX7-FKHR gene we carried out a systematic comparative analysis of changes in general cell features caused by ectopic activities of these genes. Stable cell lines expressing the corresponding proteins were derived from NIH3T3 cells and characterized in relation to the progenitor cells. These experiments revealed a dual nature of the PAX7 function. PAX7 can simultaneously be characterized as a potential tumor suppressor and a potential proto-oncogene, while its tumor suppressor-like features predominate. As a tumor suppressor-like gene PAX7 negatively controls cell proliferation, enhances contact inhibition and suppresses anchorage-independent growth. As an oncogene PAX7 prevents cells from apoptosis, inhibits cell differentiation and activates an autocrine growth stimulation. In contrast, expression of PAX7-FKHR results in neoplastic transformation: the cells loose contact inhibition, acquire anchorage-independence and gain the ability to form tumors in vivo. However, some changes in cell characteristics which are caused by PAX7 gene expression, such as autocrine growth stimulation, prevention of apoptosis and differentiation, are retained and even enhanced by expression of PAX7-FKHR. Thus, alterations of the PAX7 function in its chimeric version cannot simply be characterized as a general loss or gain of the PAX7 function. They could rather be considered as a loss of one set of functions, combined with a gain of another set of functions: the PAX7 role in negative growth control is abolished and its oncogenic features are enhanced.

Program Nr: 1851 from the 1999 ASHG Annual Meeting

***BRCA1* Analysis by DHPLC in 272 Austrian Breast Cancer only and Breast and Ovarian Cancer Families.** *T. Wagner[1], R. Moeslinger[1], E. Fleischmann[1], D. Muhr[1], M. Hareter[1], P. Kofler[1], Austrian HBOC Group[1], T. Helbich[2], C. Zielinski[3], P. Oefner[4].* 1) Dept OB/GYN, Div Senology, Univ Vienna, Vienna, Austria; 2) Dept Radiology, Univ Vienna, Vienna Austria; 3) Dept of Medicine I, Div Oncology, Univ Vienna, Vienna, Austria; 4) Dept of Biochemistry, Stanford Univ, Stanford.

In this study 272 Austrian families with hereditary breast cancer only (HBC) or breast and ovarian cancer (HBOC) were analyzed by DHPLC for sequence variants in the breast cancer gene 1 (BRCA1): In 54 (20%) HBC/HBOC families 27 different disease associated mutations were identified. Furthermore in 10 families 8 unique sequence variants of unknown functional relevance could be detected. In the total set of 275 families 32 different polymorphisms with heterozygosities of 1 of 43% were present. In 61 families (22%) with HBOC cases 25 (41%) BRCA1 mutation were identified. Ranging from 30% in families with only 1 BC to 67% in families with 3 BC cases in addition to at least 1 OC case. The overall percentage of BRCA1 mutations was 13% in the 194 (71%) HBC families. The highest percentage (26%) was observed in families with BC that occurred before the age of 30. Nine founder mutations were detected in 36 families. The most frequently detected mutation was 1806CtoT (7 families) that occurred only in Carinthia and the South of Styria. This mutation is also frequent in the south of Sweden and all families from Austria and Sweden share a common ancestor. The missense mutation 300TtoG occurred in 6 families that also share a common ancestor. This mutation is found throughout of Europe. We conclude that 40% of Austrian HBOC families and 13% of HBC families can be explained by BRCA1 mutations. More that 60% of all mutation families are identified with 1 of the 9 founder mutations in Austria. However the BRCA1 mutations detected in the remaining 18 families are scattered throughout the whole gene. A fact that makes whole gene screening mandatory in Austrian HBC/HBOC families.

Program Nr: 1852 from the 1999 ASHG Annual Meeting

Loss of heterozygosity of 5q in sporadic hemangiomas suggests that somatic mutations are involved with hemangioma development. *J.W. Walter[1], J.N. Berg[2], M. Evans[3], D. Reinhardt[2], U. Thisanayagam[2], F. Blei[4], A. Diamond[2], M. Waner[5], D.A. Marchuk[1], M.E.M. Porteous[2]*. 1) Department of Genetics, Duke University Medical Center, Durham, NC; 2) Human Genetics Unit, University of Edinburgh, UK; 3) Department of Pediatric Pathology, University of Edinburgh, UK; 4) Department of Pediatrics, New York University Medical Center, New York, NY; 5) University of Arkansas Medical Center, Little Rock, AR.

Hemangiomas are common, benign tumors of infancy which consist of rapidly proliferating endothelial cells, followed by a characteristic phase of self-involution. A locus for autosomal dominantly inherited predisposition to hemangiomas has previously been identified on chromosome 5q. We have performed microdissection of 8 sporadic hemangiomas, followed by PCR amplification and analysis of microsatellite markers on chromosomes 5 and 9. This has revealed a significant loss of heterozygosity for markers on chromosome 5q when compared to either markers from chromosome 5p ($p<0.05$) or markers from chromosome 9 ($p<0.05$). This provides evidence that a locus on 5q is also involved with sporadic hemangiomas, and suggests that formation of sporadic hemangiomas is associated with somatic mutational events.

Program Nr: 1853 from the 1999 ASHG Annual Meeting

Screening of Xq27-q28 Candidate Genes in Familial Prostate Cancer. *L. Wang[1], R.S. Berry[1], E.R. Christensen[1], A.J. French[1], D.J. Schaid[2], S.N. Thibodeau[1].* 1) Lab Medicine & Pathology, Mayo Clinic, Rochester, MN; 2) Health Sciences Research, Mayo Clinic, Rochester, MN.

Prostate cancer (PC) is the second leading cause of cancer death in the United States. Segregation analysis of familial PC suggests the presence of at least one dominant susceptibility locus, that may account for up to 10% of all PC cases. Recently, four presumed PC susceptibility loci (HPC1, PCAP, CAPB, HPCX) have been identified through linkage studies. For the HPCX region, previous linkage data, based on 123 of our PC families, indicated a locus at Xq27 that accounted for an estimated 16% of hereditary PC cases. Among the 21 polymorphic markers from Xq26-q28, we found that the peak lod scores clustered between DXS1200 and DXS1113, covering about a 2Mb region across Xq27.3 and Xq28. In an effort to clone PC susceptibility genes in this region, we constructed a physical and transcript map. Five known genes (FMR1, FMR2, IDS, U66042, MAGE9) and several unknown transcripts were identified. We also isolated a testes-specific gene (LW-1), which was localized to the distal part of our contig. This gene has a 2.2 kb transcript that encodes a protein consisting of 423 amino acids. It also has an HSF DNA-binding domain. No homologous sequences were detected in a search of public databases, suggesting that this may represent a new tumor/testes-specific protein. A total of 42 patients from 21 families were screened for variants of FMR2, U66088, MAGE-9 and LW-1 using conformation-sensitive gel electrophoresis (CSGE) and denaturing high performance liquid chromatography (DHPLC). No truncation type mutation (nonsense, frameshift, splice) was identified. However, in addition to common polymorphisms, we also detected a rare variant in the gene U66042. Mutational analysis showed a base substitution at codon 97 (GAA to AAA, Glu to Lys) in one of our 21 families. The allele frequency was 0.75% (1 of 133 normal individuals). Currently, we are analyzing additional PC families to determine whether this genetic alteration contributes to familial prostate cancer. Additionally, we are continuing to screen other candidate genes in this region.

Program Nr: 1854 from the 1999 ASHG Annual Meeting

The Establishment of a Thy-1 Inducible Expression System in Human Ovarian Cancer Cells. *N. Wang, H.R. Abeysinghe, J.E. Reeder, N.L. Guckert.* Dept Pathology, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

We have recently identified Thy-1 as a putative tumor suppressor gene for human ovarian cancer. To understand the carcinogenic role of Thy-1 and its downstream effects, a Thy-1 inducible system was established for human ovarian cancer cell line SKOV-3 based on the tetracycline (tet) regulating system originally developed by Gossen and Bujard. To establish an inducible system for the Thy-1 expression, two plasmids pTEP4m and pUHD172-1neo, which are hygromycin resistant and neomycin resistant, respectively, were co-transfected into the ovarian carcinoma cell line, SKOV-3, using the selective medium. Prior to transfection, the entire cDNA of Thy-1 was inserted into the plasmid of pTEP4m which contains the tetO-CMV operator sequence. In the plasmid pUHD172-1neo, a mutated tetracycline (tet) repressor gene, rtetR, is combined with the C-terminal domain of the Vp16 from herpes simplex virus which will lead to the production of a defected chimeric protein of rtTA. In the presence of doxycycline (dox), the rtTA will bind to the tetO and turn on the Thy-1 expression which can be reversed by the removal of doxycycline. Therefore, we can manipulate the expression of Thy-1 by doxycycline. The inducibility of Thy-1 expression in SKOV-3 cell line by dox was first confirmed at 24 hours post to dox exposure by Northern blot analysis, immunocytochemistry and flowcytometry. A time course study then revealed that the Thy-1 expression is induced 3 hours post the dox exposure and can be turned off 12 hours post removal of dox. Furthermore, both Fibronectin (Fb) and Thrombospondin were found upregulated immediately after the induced expression of Thy-1 by Northern blot analysis. In contrast, the gene SPARC was found to be independent of Thy-1 expression. In addition to the genes aforementioned, a gene discovery array screening system has been applied to the Thy-1 inducible system to identify other downstream effects of Thy-1.

Program Nr: 1855 from the 1999 ASHG Annual Meeting

HER-2/neu gene amplification in breast DCIS detected by FISH. *Z. Wang[1], L.D. Johnson[2], T. Loging[1], N. Kataoka[1], S.R. Young[1]*. 1) OB/GYN, Univ South Carolina, Columbia, SC; 2) Pathology, Univ South Carolina, Columbia, SC.

It was first reported in 1989 that amplification of the oncogene HER-2/neu is a predictor of early relapse and poor survival in breast cancer. Since that time the observation has been verified many times and extended to include a similar relationship between poor prognosis and overexpression of the HER-2/neu protein. In addition, the association between amplification/overexpression of HER-2/neu and poor prognosis has been suggested for ovarian, colon, endometrial, prostate, lung and stomach cancers. In late 1998, Herceptin, a humanized antibody against HER-2/neu, was approved by the FDA for the treatment of patients with metastatic breast cancer. Ductal carcinoma in situ (DCIS) of the breast is considered by many persons to be a precursor to invasive breast cancer. About 25% of women with untreated DCIS will go on to get invasive cancer within 10 years. The medical/surgical treatment of DCIS is dependent upon the histologic type, nuclear grade, and size of the lesion. Treatment modalities include: mastectomy, lumpectomy, and lumpectomy plus radiation, largely dependent upon the size of the DCIS area. Recurrence rates of lumpectomy with or without radiation are 10-20% within 4-5 years. We studied 30 cases of DCIS with HER-2/neu FISH to see if gene amplification was present and in what frequency. If present in sufficient numbers we could study the relationship of amplification to relapse and perhaps justify the use of Herceptin in this early stage disease. We found that 12 of 30 (40%) DCIS samples had amplification of HER-2/neu present to some degree. This high number of DCIS patients showing amplification of HER-2/neu will certainly allow study of the relationship of HER-2/neu and progression of disease and possible treatment with Herceptin.

Program Nr: 1856 from the 1999 ASHG Annual Meeting

Allele loss from chromosome 1p and from the von Hippel-Lindau locus in a patient with a sporadic carotid body tumour. *N. Weerasekera[1], P.H. Dixon[1], J. Lumley[2], C. Williamson[1].* 1) Dept. of Medicine, Imperial College, Hammersmith Hospital, London, UK; 2) Surgical Unit, St. Bartholomew's Hospital, London, UK.

Carotid body tumours can be sporadic or familial, and are inherited as either hereditary paragangliomas or as a distinct syndrome of familial carotid body tumours and multiple extraadrenal phaeochromocytomas. Hereditary paraganglioma is a genetically heterogeneous condition, with linkage reported to two loci, PGL1 (chromosome 11q23) and PGL2 (chromosome 11q13), both of which are maternally imprinted. Allelic loss of polymorphic markers from chromosome 11q has been reported in familial and sporadic paragangliomas. Allele loss in phaeochromocytomas has been reported from chromosome 1p in patients with multiple endocrine neoplasia type 2 (MEN2), and from chromosome 3p in patients with von Hippel-Lindau syndrome. Loss of tumour heterozygosity has not been reported from these loci in paragangliomas. We have performed loss of heterozygosity studies on five carotid body tumours and one glomus middle ear tumour from four patients, two of whom are from pedigrees with familial paragangliomas, one with a family history of phaeochromocytoma. We used polymorphic microsatellite markers from chromosome 11q23 (cSRL17e5(CA), cSRL17e5(CTTT), D11S1885), chromosome 11q13 (D11S480, D11S956), chromosome 3p2 (D3S1110, D3S1038) and chromosome 1p (D1S476, D1S206). In one sporadic carotid body tumour we have demonstrated allele loss from chromosome 11q23 (cSRL17e5CTTT), from chromosome 3p2 (D3S1110) and from chromosome 1p (D1S206). No loss of heterozygosity was seen in the familial tumours. This is the first report of allele loss from chromosome 3p2 and chromosome 1p in patients with paragangliomas, and suggests that genes on chromosome 1p and 3p may play a role in the multistep aetiology of these tumours.

Program Nr: 1857 from the 1999 ASHG Annual Meeting

Utility of the microsatellite instability (MSI) assay in the setting of sebaceous neoplasia and limited family history. *J.N. Weitzel[1], R. Fishel[2], K.R. Blazer[1], D.J. MacDonald[1], F.K. Fujimura[1]*. 1) Clinical Cancer Genetics, City of Hope Cancer Center, Duarte, CA; 2) Thomas Jefferson University, Philadelphia, PA.

Sebaceous neoplasms are rare. One or more sebaceous adenoma, epithelioma with sebaceous differentiation, or sebaceous carcinoma in association with visceral malignancy define Muir-Torre syndrome (MTS). MTS is an autosomal dominant genodermatosis with variable expression, including cancers of the colon, and less frequently of the stomach, uterus, ovaries, renal pelvis, or larynx. MTS is allelic to hereditary nonpolyposis colon cancer (HNPCC), which is associated with germline mutations in DNA mismatch repair (MMR) genes (primarily *MSH2* or *MLH1*). Approximately 95% of colon tumors in classic HNPCC families manifest MSI in tumor tissue. MSI is used clinically as a marker for germline MMR mutations, and MSI has been reported in sebaceous tumors from MTS families. Because sebaceous neoplasms can occur prior to or concurrently with visceral malignancy, MTS is considered in patients presenting with a sebaceous neoplasm and a positive family history of visceral cancer. The question arises as to how to assess risk for MTS in patients with cutaneous lesions and truncated or otherwise limited family histories. In this study, MSI analysis was performed on tumor specimens from five patients: two presenting with the classic MTS phenotype; one female with a personal history of breast carcinoma and sebaceous adenoma and a modest family history of GI cancers; and two males with isolated sebaceous neoplasm and negative but truncated family histories. Sebaceous tumors in two of the three isolated cases demonstrated high (>40%) MSI. One of the MSI-positive sebaceous tumors was from the female with breast cancer; her breast tumor did not demonstrate high MSI. We interpreted MSI instability as supportive of a probable MMR gene mutation and an indication for increased surveillance for visceral malignancies, and counseled patients accordingly. Germline analysis of MMR genes in the patients with MSI-positive tumors is pending. Preliminary findings suggest the possible utility of MSI testing for the refinement of cancer risk in patients with isolated sebaceous neoplasm and a limited family history.

Program Nr: 1858 from the 1999 ASHG Annual Meeting

Chromosome 8p alterations in solid tumors by array-based comparative genomic hybridization analysis. *M. Wilhelm[1], S. DeVries[1], J. Etzell[1], C. Eng[1], J. Bennington[2], A. Thor[3], J. Presti[1], F. Waldman[1]*. 1) Cancer Center, UCSF, San Francisco, CA; 2) California Pacific Medical Center, San Francisco, CA; 3) Northwestern University, Evanston, IL.

Chromosome 8p alterations have been identified in a wide range of breast cancer cell lines and primary tumors by chromosomal comparative genomic hybridization (CGH). Deletions of distal 8p combined with gains or amplifications of proximal 8p were seen in 20-50% of breast tumor samples. By chromosomal CGH, the transition point between distal loss and proximal gain can be mapped to band 8p12. However, resolution of this approach is limited in the metaphase chromosome to approximately 10 Mb, making further delineation of this candidate region difficult. An array-based CGH approach increases resolution by hybridizing the tumor and reference DNAs onto a collection of selected target clones. Resolution is thus limited only by the proximity of the clones to each other. In this study, CGH-array analysis was performed with using 20 clones (BAC and P1) spanning chromosome 8. Array-based analyses generally agreed with CGH profiles for chromosome 8 in our samples. One bladder cell line (TCC) showed loss encompassing the whole 8p arm, one kidney cell line (769-P) showed gain of the entire 8q arm, while two cell lines, one renal and one breast (786-0, BT474), showed an associated loss of 8p and gain of 8q. An additional three breast tumor cell lines and 4 primary tumors showed partial loss of chromosome 8p, with a distal deletion starting at band 8p12. Array-based CGH showed these alterations at high resolution, and was able to map a breakpoint within band 8p12. Two DNA clones (RMC08P047, RMC08B2141) were identified adjacent to this transition, with the proximal one showing an amplification. Interestingly, three of the primary breast cancers were well differentiated tubular carcinomas. For these samples, the array analyses were done using DOP-PCR amplified DNA extracted from paraffin sections after microdissection. In conclusion, array-based CGH yields high resolution analysis of cytogenetic aberrations in primary tumor samples, aiding positional cloning of candidate genes.

Program Nr: 1859 from the 1999 ASHG Annual Meeting

A Functional Genomic Approach for the Identification of *PAC-1*, a Novel Chromosome 10 Tumor Suppressor Gene. *P.E. Wong, M.M. Lovell, A. Goodacre, A.M. Killary.* Pathology and Laboratory Medicine, UT M.D. Anderson Cancer Center, Houston, TX.

A major goal of our laboratory's research is to use a functional genomic approach to define and isolate tumor suppressor genes involved in prostatic adenocarcinoma. Previously, we have defined a novel tumor suppressor locus *PAC-1* within chromosome 10p. Introduction of the short arm of chromosome 10 into a prostatic adenocarcinoma cell line PC-3H resulted in dramatic tumor suppression and restoration of a programmed cell death pathway. To functionally dissect the region within 10p containing *PAC-1*, we developed the novel strategy of serial microcell fusion, a technology that would allow the transfer of defined fragments of chromosome 10p into PC-3H and the rapid *in vivo* assay for functional tumor suppressor activity. Serial microcell fusion was used to transfer defined 10p fragments into a mouse A9 fibrosarcoma cell line. Once characterized by FISH and microsatellite analyses, the 10p fragments were subsequently transferred into PC-3H to generate a panel of microcell hybrid clones containing overlapping deletions of chromosome 10p. Additionally, comparative genomic hybridization was used to identify regions of loss in PC-3H. Extensive characterization of 10p deletion hybrids by microsatellite analysis and FISH allowed the exclusion of two separate candidate tumor suppressor regions within chromosome 10p implicated by LOH in gliomas and prostate tumors. Furthermore, these data limited the region for *PAC-1* from approximately 65 Mb to less than 10 Mb. These studies demonstrate the utility of this approach as a powerful tool to limit regions of functional tumor suppressor activity. In addition, the 10p deletion hybrid panel will be a critical resource for the physical mapping of 10p, as well as for the isolation of the important tumor suppressor gene *PAC-1*.

Program Nr: 1860 from the 1999 ASHG Annual Meeting

Tumour Formation in Neurofibromatosis 2 (NF2): a test of fit for a "two-hit" hypothesis. *R. Woods[1,5], D.G.R. Evans[2], H. Joe[1,5], M.E. Baser[3], J.M. Friedman[1,4].* 1) University of British Columbia,Vancouver, British Columbia, Canada; 2) St. Mary's Hospital, Manchester, U.K; 3) Los Angeles, U.S.A; 4) Department of Medical Genetics; 5) Department of Statistics.

Hethcote and Knudson (Proc Natl Acad Sci 1978;75:2453-7) developed a "two-hit" model to describe the incidence of tumours in hereditary and sporadic retinoblastoma. Subsequent molecular genetic analysis has established the validity of this model in retinoblastoma and other inherited forms of cancer. Molecular evidence suggests that a two-hit model is also appropriate for the development of vestibular schwannomas (VS) in patients with neurofibromatosis 2 (NF2). We fit a model analogous to Hethcote and Knudson's to incidence data for sporadic and inherited VS, the latter in NF2 patients, to examine the correlation of epidemiological and molecular evidence in this condition. The data included 72 NF2 patients, all non-probands with bilateral VS, and 50 published sporadic cases of unilateral VS (Clin. Otolaryngol 1999;24:13-18). The age at onset of VS in sporadic cases is later than that in patients with NF2 (mean/median age of onset in years, 52.1/56.5 for sporadic cases, 30.1/26.0 for NF2 cases). The predicted incidence curves for the age at onset of VS from the two-hit model fit the empirical incidence curves very closely. (chi-square(8 df) = 4.59 for goodness of fit, p = 0.80)(See http://mendel.medgen.ubc.ca/friedmanlab/2hitplot.html for the plot). We are extending this model to include provision for the allele-phenotype correlation that has been described in NF2 (e.g., J Med Genet 1998;35:450-455) and to analyze the occurrence of other tumours in this disease. The authors gratefully acknowledge support from the Acoustic Neuroma Association of Canada, the BC Medical Services Foundation, and NSERC.

Program Nr: 1861 from the 1999 ASHG Annual Meeting

Risk of breast cancer from benign breast disease: Incidence Rates for breast cancer and intra observer reliability in classifying benign breast disease lesions. *M.J. Worsham[1], C.C. Johnson[2], U. Raju[1], J. Abrams[2], A. Blount[2], S.R. Wolman[3]*. 1) Cancer Genetics Research, Dept Pathology, Henry Ford Hosp, Detroit, MI; 2) Josephine Ford Cancer Center, enry Ford Hosp, Detroit, MI; 3) Uniformed Services University of the Health Sciences, Bethesda, MD 20814-4799.

Women with benign breast lesions, particularly those classified as proliferative, are at increased risk for subsequent development of breast cancer. We have identified a cohort of women with benign breast disease (BBD) diagnosed by breast biopsy during the years 1981-1994. Classification was based on risk categories for developing invasive carcinoma defined by Page and Dupont. At the current time, 2263 members of the cohort have been followed for occurrence of breast cancer through 1997. One hundred thirty one cases were identified over 21,317 person-years of follow-up. The average incidence rate per year was 615 per 100,000 (95%) confidence interval of 518-729). The incidence of breast cancer in this BBD cohort appears to be higher than the SEER rate of 350.2 per 100,000 for women 50 years from 1990-1994 in the same metropolitan areas. To evaluate intra observer reliability, a 10% random sample of slides, N=74, from years 1981 through 1983 were independently reviewed a second time by the same pathologist who was blinded at both readings to the identity of the patient. Concordance ranged from 85% for simple apocrine metaplasia to 99% for squamous metaplasia. Average agreement was 91%. Kappa statistics indicated significantly greater than chance agreement ($p<.001$) for all lesions but fibrosis. Concordance ranged from 93% for simple adenoid and hyperplasia to 99%for apocrine hyperplasia with a mean of 96%. All kappa statistics indicated significantly more than chance agreement, $p<.001$. Lesions with moderately increased risk are atypical ductal hyperplasia (ADH) and atypical lobular hyperplasia (ALH). One case of ADH and no cases of ALH were found and the pathologist agreed at both readings. No high-risk lesions i.e. ductal or lobular carcinoma in situ were found. In summary, a trained breast pathologist can reliably classify lesions of different risk categories.

Program Nr: 1862 from the 1999 ASHG Annual Meeting

HER2/neu and PS6K (17q23) amplification in breast lesions from women with and without a family history of breast cancer. *G. Wu, K. Anderl, C.A. Soderberg, L.C. Hartmann, R.B. Jenkins, F.J. Couch.* Mayo Clinic, Rochester, MN.

Germline mutations in BRCA1, BRCA2, and other susceptibility loci are involved in the initiation of hereditary breast cancer. However, the somatic events that lead to progression of hereditary cancers are not known. In addition, it is not known whether somatic progression pathways differ in hereditary versus sporadic disease. To address this question we have evaluated the frequency of amplification of HER2/neu and a region of chromosome 17q23 containing the PS6K (p70 S6 kinase) gene in a series of 22 cases of breast cancer from families with strong histories of breast and/or ovarian cancer and 29 matched sporadic cases. Paraffin blocks for 46 in situ carcinomas and 51 invasive carcinomas were identified for these cases, along with 32 regions of apparently benign epithelium (for control purposes). These blocks were subjected to fluorescent in situ hybridization (FISH) analysis using HER2/neu/CEN17 and PS6K/CEN17 dual-BAC probe mixtures.

The incidences of gene amplification in hereditary and sporadic cases, respectively, were 28% (6 of 21) and 0% (0 of 13) for HER2/neu, and 44% (7 of 16) and 25% (3 of 12) for PS6K. The incidences of chromosomal gain in hereditary and sporadic cases, respectively, were 65% (13 of 20) and 38% (5 of 13) for +CEN17. In hereditary cancers 1 of 16 had co-amplification of HER2 and PS6K, 3 of 16 had amplification of HER2 only, and 6 of 16 had amplification of PS6K only. In sporadic cancers, 0 of 12 showed co-amplification, 0 of 12 had amplification of HER2 only, and 3 of 12 had amplification of PS6K only. Our data show that chromosomal alterations, as measured by CEN17 gain and HER2/neu and PS6K gene amplification, are more frequent within breast cancers of women with a strong family history of cancer, consistent with an increased underlying chromosomal instability. Further studies will determine if separate pathways of carcinogenesis exist for hereditary and sporadic breast cancer.

Program Nr: 1863 from the 1999 ASHG Annual Meeting

Localization of Transfected B7-1 (CD80) DNA on Human Melanoma Cells after Particle Mediated Gene Transfer. *S. Wu[1], D.O. McCarthy[2], N.J. Glowacki[2], C.A. Emler[3], X. Chen[1], M.R. Albertini[2].* 1) Pediatrics, Children's Hospital, Los Angeles, School of Medicine, University of Southern California, Los Angeles, CA; 2) University of Wisconsin Comprehensive Cancer Center, Madison, WI; 3) Agracetus, Inc., Middleton, WI.

Particle mediated gene transfer (PMGT) is an efficient means to directly deliver transgenes into human melanoma cells for transient or stable gene expression. The aim of this study was to determine the localization of transfected B7-1 cDNA in M-21 melanoma cells following PMGT. Microscopic gold particles coated with a plasmid vector containing B7-1 cDNA and delivered by PMGT were detected in the cytoplasm and nucleus of recipient M-21 cells. Using fluorescence in situ hybridization (FISH), biotin labeled B7-1 DNA was clearly detected in most melanoma cells 24 hours following PMGT. The B7-1 transgene particles were randomly distributed within melanoma cells, and transfected melanoma cells contained between 1 and 14 biotin labeled particles. Stable B7-1 transfectants (M-21-B7) were obtained following PMGT with a plasmid vector containing cDNA for both B7-1 and neomycin phosphotransferase and subsequent selection in media containing G418. While 50-60% of M-21-B7 evaluated by flow cytometry had surface expression of B7-1, analysis by FISH did not detect the B7-1 transgene in these cells. The M-21-B7 were then sorted on the FACStar Plus by brightness of B7 expression to obtain M-21-B7 (bright) cells with 85-90%of cells with B7-1 expression. Analysis by FISH and subsequent chromosome G-banding analysis demonstrated 70% of M-21-B7 (bright) to have two predominant integration sites with extensive amplification of the B7-1 transgene. These integration sites were located on chromosome 15 and 17. These findings demonstrate that B7-1 cDNA can integrate into the chromosomes of recipient M-21 cells following PMGT, and this integration can occur in preferential sites in some cells. The relationship between transgene integration site and expression of the transgene, or other cellular genes, requires further investigation.

Program Nr: 1864 from the 1999 ASHG Annual Meeting

Genomic organization and mutation analysis of Mortalin, a candidate for the chromosome 5q31 tumor suppressor gene in AML/MDS. *H. Xie[1], S.K. Horrigan[2], B. Chyna[1], Z.B. Hu[1], C.A. Westbrook[1]*. 1) Section of Hematology/Oncology, Univ. of Illinois at Chicago, Chicago, IL; 2) Department of Pediatrics, Lombardi Cancer center, Georgetown University Medical Center, Washington,DC.

In malignant myeloid disorders, including myelodysplasia (MDS) and acute myeloid leukemia (AML), interstitial deletion or loss of chromosome 5 frequently occurs, suggesting the presence of a tumor suppressor gene. By heterozygosity analysis of a small deletion, we have defined a minimal localization for this gene to a 700 kb interval on 5q31, to which we have mapped several candidate genes. Among these candidates, mortalin, HSPA9,is a plausible tumor suppressor. Mortalin is a member of the hsp70 family. The protein exhibits differential cellular localization in mortal and immortal cells of human and mouse by virtue of its cytosolic and perinuclear distribution, respectively. The murine cytosolic form (mot-1) induces senescence in NIH3T3 cells whereas the perinuclear form (mot-2) does not. It has been postulated that loss of cytosolic mortalin may lead to cell immortalization and tumorigenesis. As first step in mutation analysis of mortalin in clinical AML and MDS, we determined the genomic structure and intron-exon boundaries of human mortalin by direct sequencing of BAC DNA. We show that the gene spans 19.5 kb. It contains 17 exons and 16 introns, with similar boundaries to its murine counterpart, and all boundary sequences contained consensus GT/AG sequences at the donor and acceptor site of RNA splicing. Mutation analysis of AML was begun by sequencing DNA from three AML cell lines, including 2 with chromosome 5 loss (KG-1 and HL-60) and one without (AML-193). Using intron-based primers, genomic sequence was completed for all 17 exons, and compared to normal (BAC) DNA. No mutation was detected in any of the cell lines, although two conservative nucleotide sequence variants were identified in exon 16. We have shown by RT-PCR that mortalin is expressed in these AML cell lines and normal CD34+ bone marrow precursor. Mutation and expression analysis of clinical MDS and AML samples is ongoing.

Program Nr: 1865 from the 1999 ASHG Annual Meeting

Identification of differetially expressed genes by reciprocal subtraction cDNA array screening. *J. Xu, H.R. Abeysinghe, N. Guckert, N. Wang.* Dept Pathology, Univ Rochester Medical Ctr, Rochester, NY.

A reciprocal subtraction cDNA array screening approach has been applied to identify the differentially expressed genes associated with suppression of tumorigenicity in the human ovarian carcinoma cell line SKOV3. The non-tumorigenic subclones 11(H)7-2 and 11(H)9-8 and slow growing tumorigenic subclone 11(H)8-3 were derived from SKOV3 with the introduction of human chromosome 11. Reciprocal subtraction of cDNAs between tumorigenic and non-tumorigenic subclones followed by gene discovery array screening revealed that 15 known genes and 6 ESTs were either absent or downregulated in the two non-tumorigenic subclones , but were expressed in the slow growing tumorigenic subclone . In contrast, 81 known genes plus 59 ESTs were either absent or downregulated in the slow growing tumorigenic subclone , but were expressed in the two non-tumorigenic subclones . Four of these 81 knonw genes, CRYAB, IGSF4, THY-1, and APLP2, are located at the band 11q23. Structural alterations at this chromosomal region are frequently identified in human ovarian cancer. Therefore, 11q23 may be a critical region for human ovarian cancer.

Program Nr: 1866 from the 1999 ASHG Annual Meeting

Myeloperoxidase Genetic Polymorphism and Colorectal Adenoma Risk. *W.M Xue[1], R.W Haile[1], H.D Frankl[2], E.R Lee[3], S.A. Ingles[1]*. 1) Preventive Medicine, University of Southern California, Los Angeles,CA; 2) Division of Gastrienterology, Sunset Kaiser Permanente Medical Center, Los Angeles, CA; 3) Division of Gastroenterology, Bellflower Kaiser Permanente Medical Center, Los Angeles, CA.

Myeloperoxidase (MPO) is a neutrophillic enzyme which catalyses the reaction of chloride and hydrogen peroxide to yield hypochlorous acid, a strong oxidant. A G/A polymorphism in an Alu repeat in the MPO gene promoter has been shown to be functionally significant in vitro, with the presence of a G rather than an A at this site increasing gene expression. We used an RFLP/PCR method to genotype subjects from a sigmoidoscopy screening program in two Southern California Kaiser Permanente medical centers.This study includes a control group of 474 subjects without adenomas, a case group of 302 subjects with left colon adenomas and 131 with rectal adenomas. The ethnic breakdown was 52% White, 17% African-American, 18% Hispanic and 12% Asian. Among Hispanics and non-Hispanic Whites, 3% of subjects carried 2 copies of the A allele. Whereas 15% of African-Americans and 1% of Asians had the AA genotype. Among African-Americans, the AA genotype was associated with a more than two-fold increase in risk of colorectal adenomas (OR = 2.83, 95% confidence interval, 1.02-7.82; P = 0.04). Risk was similarly elevated for rectal and left colon adenomas. However, the AA genotype was associated only with rectal adenomas in other ethnic groups (OR = 3.05, 95% confidence interval, 1.11-9.07; P = 0.03). Possible explanations for this finding will be discussed.

Program Nr: 1867 from the 1999 ASHG Annual Meeting

A unique clone involving multiple structural chromosome rearrangements in a myelodysplastic syndrome case. *J. Yan[1], R. Whittom[2,3], R. Drouin[1]*. 1) Pathology, Dept Medical Biology; 2) Hematology, Dept Medical Biology; 3) Hematology, Dept Medicine, Laval University, Pav St-Francois d'Assise, CHUQ, Quebec, Quebec, CANADA.

Cytogenetic abnormalities are present in more than 50% of myelodysplastic syndrome (MDS) patients. Cytogenetic studies using bone marrow samples add important diagnostic and prognostic information to this hematological disorder. The clinical prognosis of MDS is greatly affected by karyotype complexity. The patients having a normal karyotype or only one chromosomal abnormality usually predict a better prognosis. On the other hand, patients having complex chromosomal aberrations (more than two abnormalities) are often associated with the worst prognosis (a higher risk of acute leukemia transformation and a shorter survival). We identified a unique clone involving multi-chromosome rearrangements in a 40-year old female patient suffering from refractory anemia with ringed sideroblasts. Fifty G-banded metaphases were initially analyzed. All of the 50 metaphases showed six abnormal chromosomes including two ring chromosomes and other aberrations that are not usually found in MDS. The initial karyotype was defined as: 46,XX,-5,-11,add(11)(p15),del(13) (q14),add(20)(p13),idic(22)(p11),+r1,+r2. To further identify these chromosome abnormalities, we used molecular cytogenetic methods, such as FISH (fluorescence in situ hybridization) and PRINS (PRimed IN Situ labeling). Finally, the karyotype was defined as: 46,XX,r(5)(p13q13), t(5;20)(p13;p13),r(11)(p15q25),dup(11)(p11.2p15), del(13)(q14),idic(22)(p11). The patient quickly progressed to an acute non-lymphocytic leukemia (ANLL) three months following cytogenetic diagnosis and died of a hemorrhage in the brain parenchyma two months later. The very poor prognosis was predicted by the multiple (six) structural chromosome rearrangements and by the types of rearrangements. We concluded that, for this case, the most important chromosome abnormalities were the 5q- and 13q14- (Rb gene deletion). Such rearrangements can be closely correlated to very poor prognosis of the rapid leukemic transformation, the lack of response to treatment and the short survival.

Program Nr: 1868 from the 1999 ASHG Annual Meeting

Absence of SH2D1A point mutation in 62 Burkitts lymphoma cell lines. *L. Yin, T. Tocco, S. Pauly, G.M. Lenoir, G. Romeo.* International Agency for Research on Cancer, Lyon, France.

X-linked lymphoproliferative disease (XLP) is a rare inherited immunodeficiency characterized by selective susceptibility to Epstein-Barr Virus (EBV). The recent identification of the XLP gene, SH2D1A, revealed a EBV-related signal transduction pathway (Coffey et al., Nature Genet. 20:129-135, 1998; Sayos et al., Nature 395:462-469, 1998), which is not only essential in understanding the mechanism of XLP, but also will give some insights into other EBV-associated diseases, including Burkitts lymphoma (BL). BL is characterized as a small, non-cleaved cell lymphoma (SNCL), invariably of B cell origin. Monoclonal EBV DNA is found in virtually all cases of endemic BL in Africa and in a significant part of sporadic BL from other regions. The common feature of XLP and BL is the uncontrolled proliferation of the EBV infected cells. In addition, one of the typical phenotypes of XLP is that the patients often develop malignant lymphomas of the Burkitts type. We hypothesize that a defect of SH2D1A gene, which regulates the signal transduction cascade(s) relevant to the immune response to EBV infection, might be responsible for a certain proportion of BL cases. DNA from 62 BL cell lines were screened for mutations by PCR amplification and sequencing, using primers reported by Coffey et al. 1998. In the coding region of all the four exons of SH2D1A, in the 5 non-translated region, part of the 3 non-translated region, as well as in approximately 50 bp of intronic sequences flanking each exon, no point mutation was present in these 62 BL patients. This result suggests that missense, nonsense, splicing mutations of the SH2D1A gene are not the major cause for this disease. However, it can not be excluded that an altered expression level of the SH2D1A gene may relate to BL.

Program Nr: 1869 from the 1999 ASHG Annual Meeting

Molecular cytogenetic characterization of ovarian carcinoma, III: 20q. *S.R. Young[1], Z. Wang[1], S.T. Smith[1], R.S. Parrish[2]*. 1) Dept OB/GYN, Univ South Carolina Sch Med, Columbia, SC; 2) S.C. Cancer Ctr, Univ South Carolina Sch Med, Columbia, SC.

Ovarian cancer is the leading cause of gynecologic cancer death in the U.S. This high mortality is largely because the ovaries are located deep in the pelvis and the malignancy is not diagnosed until there is an advanced stage of disease. It is important to better understand the biology dictating the etiology and progression of ovarian cancer to facilitate earlier diagnosis and better treatment. In 1989, using Southern blot methodology, it was shown that amplification of the oncogene HER-2/neu was a poor prognostic indicator in ovarian cancer. Overexpression of p53 is found in some ovarian carcinomas but not in benign tissue. More recently serum lysophosphatidic acid was reported to be a biomarker of disease. Pelvic ultrasound and serum CA125 studies have proven inefficient in early diagnosis of ovarian cancer. From Jan 1993 through May 1995 we collected 40 ovarian cancer surgical samples. We have previously described the results of our FISH studies of HER-2/neu and centromere #17, c-myc and centromere #8 and p53 on these samples. Briefly, 10/40 (25%) had amplified HER-2/neu, 16/40 (40%) had amplified c-myc, and 9/40 (22.5%) had deletion of p53. Only those patients whose tumors were amplified for both oncogenes had a statistically significant shorter survival. We now report the addition of FISH studies of 20q13 conducted on these very same samples. Of the 40 samples studied, 4 (10%) showed medium/high amplification of 20q13. There was no significant correlation between these results and the patient's clinical findings. It is of interest that in every sample with amplified 20q13 there was also amplification of c-myc (Fisher's exact p-value= 0.020). This data suggests that there should be further study of the relationship between amplification of 20q13 and c-myc in ovarian cancer samples.

Program Nr: 1870 from the 1999 ASHG Annual Meeting

Detection of homozygous deletions in tumors by hybridization of representational difference analysis (RDA) products to chromosome specific YAC clone arrays. *M. Zeschnigk, B. Horsthemke, D.R. Lohmann.* Institut für Humangenetik, Universitätsklinikum Essen, Hufelandstrasse 55, 45122 Essen, Germany.

Biallelic inactivation of tumor suppressor genes is frequently accompanied by loss of genetic material. Therefore, identification of deleted regions in tumor genomes is an important step towards the isolation of these genes. Subtractive hybridization techniques facilitate to enrich differences between complex genomes and can help to identify homozygously deleted regions. The enrichment efficiency was improved when Lisitsyn et al. introduced the RDA. As this PCR based technique also enriches polymorphic fragments that are lost in driver representation due to chromosomal rearrangements that result in loss of heterozygosity (LOH) laborious clone by clone analysis is required because typically only a few percent of the cloned RDA products are derived from the homozygous deletion. To overcome this limitation, we deviced a novel strategy in which difference products generated by RDA are labeled and hybridized in toto against an ordered array of YAC clones covering a region of interest. The YAC clones identified by the RDA products provide positional information on homozygous deletions and LOH regions. To demonstrate the usefulness of the approach, we have tested this strategy by analyzing a retinoblastoma with a known homozygous deletion affecting the RB1 gene and LOH at neighbouring polymorphic loci on chromosome 13. RDA was performed using representations generated with restriction enzymes BglII, NcoI and XbaI, and the products of each RDA experiment were hybridized to chromosome 13 YAC filters. The map positions of YACs identified by RDA products of a single restriction enzyme were scattered over the whole chromosome with a slight accumulation in the LOH and RB1 region. However, when collating the information from three different RDA experiments only one region which comprised the homozygously deleted region on chromosome 13 showed a cluster of hits. In summary, the combination of the RDA with direct hybridization of the RDA products to chromosome specific YAC clone filters may facilitate to scan multiple tumors for homozygously deleted regions in the presence of LOH regions.

Program Nr: 1871 from the 1999 ASHG Annual Meeting

Role Of The p53 and PTCH Genes in Early Onset Basal Cell Carcinoma. *H. Zhang, X.L. Ping, P.K. Lee, X.L. Wu, Y.J. Yao, M.J. Zhang, F.F. Chen, D.N. Silvers, D. Ratner, M. Peacocke, H.C. Tsou.* Dermatology, Columbia University, New York, NY.

Basal cell carcinoma (BCC) is the most common skin cancer in the Western world. UVB irradiation from sunlight is the major risk factor for the development of BCC. The incidence of sporadic BCC increases in individuals over age 55, with the greatest incidence reported in individuals who are over 70. The incidence of BCC is rare in individuals who are younger than 30. Two tumor suppressor genes, p53 and PTCH, have been implicated in the development of BCC. Mutations in the p53 have been found in 30 to 50% of BCC studied. Somatic mutations in the PTCH gene were identified in 20 to 30% of the sporadic BCC studied. Most of mutations detected in these two genes were UV-specific C->T or CC->TT changes. In this study, we analyzed 23 BCC samples from individuals who have BCC diagnosed by age of 30. They are all Caucasians, and age ranged from 16 to 29 years old. Genomic DNA from each BCC was screened for mutations in the PTCH gene by PCR-SSCP. Seventeen SSCP variants in the PTCH gene were identified from 14 BCC samples. Sequence analysis of these SSCP variants revealed 15 single nucleotide changes, one AT insertion after nucleotide 2709 in exon 7 and one 15 bp deletion after nucleotide 2385 in eoxn 15. Most of these nucleotide changes (9/17) were predicted to result in truncated PTCH proteins. The same 23 BCC samples were then screened for mutations in the p53 gene by complete sequence analysis. Fifteen p53 mutations were found in 11 BCC samples. They consisted of 11 missense mutations, one nosense mutation and three deletions. Over 60% of these nucleotide changes were UV specific. Interestingly, p53 mutations were only found in BCC samples containing PTCH mutations. Our data demonstrated that both p53 and PTCH are implicated in the development of early onset BCC. The identification of the UV specific nucleotide changes in both tumor suppressor genes suggests that UV exposure is an important factor in the early onset BCC.

Program Nr: 1872 from the 1999 ASHG Annual Meeting

DEVELOPMENT OF NEW MOLECULAR GENETIC MARKERS OF OUTCOME IN PEDIATRIC EWING GROUP TUMOUR. *Z.M. Zhang[1], Y.K. Ng[2], J. Bayani[1], P. Marrano[1], M. Zielenska[3], J. Squire[1]*. 1) Cellular & Molecular Biology, Ontario Cancer Institute,University Health Network, Toronto, Ontario, Canada; 2) Cytogenetics Program, Banting Institute, University Health Network, Toronto, Ontario, Canada; 3) Department of Pediatric and Laboratory Medicine, Hospital for Sick Children, Toronto, Ontario, Canada.

Ewing sarcoma (ES) is a bone tumour of childhood in which there is variable response to treatment. ES can be aggressive with a high propensity for local recurrences and distant metastases. In contrast, other children have a very favorable response to therapy and have no subsequent disease complications. This study is focusing on developing prospective panel of cytogenetic markers that will prognosticate for those ES tumours in which advanced disease and tumour recurrence is more likely to occur. Our approach is to use a combination of molecular cytogenetic techniques such as Fluorescence in situ Hybridization (FISH) on paraffin section, Comparative Genomic Hybridization (CGH) and Spectral Karyotyping (SKY) to augment classical cytogenetics, and define a pattern of chromosomal change that will predict which tumours are more aggressive. We present a study of ES tumors in which a more complex pattern of chromosomal change is present and is associated with those tumors that fail to respond to treatment. These include 30 ES tumors derived from paraffin sections by interphase FISH and correlated presence or absence of trisomies 8 and 12 with disease response. In prospective non-responders intervention may be considered earlier, so that intensified treatment regimens can be carried out at an earlier phase in the disease process.

Program Nr: 1873 from the 1999 ASHG Annual Meeting

Variant *NPM-ALK* chimeric product in the anaplastic large cell lyphoma (ALCL). *M. Zielenska[1], W.L. Sirkin[1], N. Fabricius[1], J. Zielenski[2]*. 1) DPLM, The Hospital for Sick Children, Toronto, Ontario, Canada; 2) Department of Genetics, The Hospital for Sick Children, Toronto, Ontario, Canada.

Chromosomal translocations found in neoplasms often result in the creation of hybrid genes encoding chimeric proteins. Anaplastic large cell lymphoma (ALCL) is a distinct clinico-pathologic variant best classified as a high grade non-Hodgkins lymphoma (NHL) composed of large pleomorphic cells that usually express the CD30 antigen and interleukin (IL)-2 receptor. With variable frequency ALCL bears the t(2;5)(p23;q35) chromosomal translocation that fuses the nucleoplasmin (*NPM*) gene on chromosome 5q35 to a novel protein kinase gene, anaplastic lymphoma kinase (*ALK*) on chromosome 2p23. We routinely use RT-PCR method for detection of *NPM-ALK* PCR chimeric mRNA transcripts. In all but one case the *NPM-ALK* RT-PCR products were of identical size, suggesting that the genomic chromosome breaks are clustered in a single intron in both *NPM* and *ALK* genes. The single exception was found in an eight-year old patient with a T-lineage, CD30+ ALCL that expresses a larger (+84 basepair) *NPM-ALK* chimeric product. The consequence of this new variant for *NPM-ALK* is at present unclear. Additional cases will need to be characterized to determine whether this variant translocation carries any significance with respect to clinical features, or prognosis.

Program Nr: 1874 from the 1999 ASHG Annual Meeting

Mosaic tetrasomy 3q25®ter. *M.L. Levin[1], T.R. Dennis[2], P. Spallone[2], A.D. Stock[2].* 1) Pediatrics, Genetics Division, U. Nevada School of Medicine, Las Vegas, NV; 2) Pathology, Molecular Cytogenetics Laboratory, U. Nevada School of Medicine, Reno, NV.

We report the first case of mosaic tetrasomy 3q25®ter and discuss the findings in relation to the 3q duplication syndrome, Cornelia de Lange syndrome, and the Opitz-C syndrome which have been reported to have a superficial resemblance to 3q duplication syndrome. The patient has a de novo 47,XX,+der(3)iso(3)(q25)/46,XX, with the level of mosaicism only 5% in peripheral blood and more than 90% in skin. The derived chromosome's origin was confirmed by microdissection and reverse in situ hybridization. The patient has post-natal growth retardation and is functioning at the 8-9 month level when last seen at 16 months of age. Hypopigmented "striping" of the nose and glabella noted at birth has progressed to a whorled pattern covering most of the body. Dysmorphic facial features include sparse hair, dolichocephaly, narrow nose with pointed tip and anteverted nares, brushfield spots, epicanthal folds and upslanting palpebral fissures, retrognathia, high arch palate, inverted cupid's bow mouth and low set rotated ears. Bilateral styes have developed. Skeletal findings include hip dislocation, sacral vertebral abnormalities, clinodactyly with overlapping index and 5th fingers, single palmar and aberrant flexion creases, and short toes. The patient has labial hypoplsia and severe hydronephrosis which resulted in intestinal obstruction in the neonatal period. Evaluation revealed bilateral ureteropelvic junction obstruction, worse on the right with an ectopic ureteral insertion into the urethra. Percutaneous right nephrostomy with cutaneous pyelostomy were performed. Comparison with other patients with duplication 3q is not straightforward because of mosaicism and tetrasomy; there is one report of mosaic trisomy 3q and all other reports describe trisomy for various segments of 3q, many having other partial aneusomies as a result of unbalanced translocations. Our patient has developmental delay, growth retardation, facial features and genitourinary anomalies common to some patients with 3q duplication , but does not resemble the Cornelia de Lange syndrome or C syndrome phenotypes.

Program Nr: 1875 from the 1999 ASHG Annual Meeting

The product of the Hira gene, a potential chromatin factor, is required for early mammalian differentiation and proliferation. *F.P. Lohman[1], V. Lui[1], S. Snoeren[1], P. Scambler[2], F. Grosveld[1], C. Meijers[1].* 1) Cell Biology & Genetics, Erasmus University, Rotterdam, The Netherlands; 2) Institute of Child health, London.

The Hira gene is a candidate gene for the DiGeorge syndrome, which is characterized by defective development of pharyngeal arches and haplo-insufficiency for chromosome 22q11. The Hira protein shares significant homology with the p60 subunit of the human chromatin assembly factor I (CAF-I) and two co-repressors of gene transcription in yeast, Hir1p and Hir2p. It has been shown that members of the chromatin remodeling Swi/SNF complex in yeast binds strongly to Hir1p and Hir2p. Hira homologues have been identified in mouse, chicken, frog, worm and fly suggesting a fundamental role of the Hira protein in both vertebrates and invertebrates. High levels of Hira mRNA are present in embryonic structures that are affected in 22q11 deletion syndrome patients. Knockout of Hira in mice is lethal at E9.5 and embryonic development in most embryos is arrested at the gastrulation stage. Heterozygous and homozygous mutant embryonic stem cells are viable, indicating that the Hira gene is not essential for cell viability. The cell cycle in these Hira mutants is shortened, while their differentiation is impaired. Introduction of a tagged full size Hira cDNA to -/- ES ells rescues the proliferation phenotype.GFP-tagged Hira locates to the nucleus, cytoplasm or both. This indicates a cell cycle dependent localization. Truncated versions of GFP-Hira constructs indicate that the domain responsible for cytoplasmic localization resides in the NH2 domain. Immunohistochemistry of mouse and human bone marrow and peripheral blood shows that the endogenous Hira protein is excluded from the nucleus in terminally differentiated cells. These results suggest that the Hira protein is required for cellular differentiation and proliferation during early mammalian development and implicate a role of Hira during the cell cycle. A quantitative defect in HIRA production could translate in defects in chromatin remodeling activities, a possible basis for some of the phenotypic features in 22q11 deletion syndrome patients.

Program Nr: 1876 from the 1999 ASHG Annual Meeting

Thymic hypoplasia and multiple malformations in the child of a liver transplant recipient. *M.A. Lovell, L.B. Karns, J.E. Ferguson, M.L. Buck, W.G. Wilson.* Depts. of Pathology, Obstetrics and Gynecology, and Pediatrics, Univ of Virginia Health System, Charlottesville, VA.

We report on a 3 month old girl, born to a 22 year old woman with Alagille syndrome 12 years status post orthotopic liver transplantation, who died from sepsis and complications related to multiple congenital malformations. The 1550 gm infant was born at 33 weeks gestation following a pregnancy complicated by polyhydramnios and maternal medication exposures (tacrolimus, prednisone, and lamotrigine). She died following a 3 month NICU course. Autopsy findings included a type II tracheoesophageal fistula, duodenal atresia, thymic hypoplasia with absence of thymocytes, systemic lymphoid depletion, agenesis of the upper and middle lobes of the right lung, absent upper right pulmonary vein, and segmental bronchomalacia. The liver did not show evidence of Alagille syndrome. A lymphocyte karyotype was normal (46,XX). The malformations present in this child suggest a developmental field defect, and are not typical of those seen in Alagille syndrome. To our knowledge, this pattern of malformations has not been previously reported in association with maternal exposure to these medications. Tacrolimus is a relatively new macrolide antibiotic with potent immunosuppressant properties, and has not been associated with an increased incidence of malformations, although prematurity, transient anomalous renal function, and dilated cardiomyopathy have been reported. Lamotrigine, a phenyltriazine anticonvulsant, is also relatively new and has not been associated with this pattern of malformations. Whether the findings in this infant are coincidental, related to Alagille syndrome, or are related to the medications taken by the mother cannot be determined at this time.

Program Nr: 1877 from the 1999 ASHG Annual Meeting

A clinical study of patients with isolated multiple neurofibromas. *M. MacCollin[1], P. Blakley[2], D. Louis[3], H. Mankin[4], M.P. Short[5]*. 1) Dept Neurology; 2) Genetics and Teratology Unit; 3) Dept Pathology; 4) Dept Orthopaedics, Massachusetts General Hospital, Boston, MA; 5) Dept Neurology, University of Chicago, Chicago, IL.

Neurofibromatosis Type 1 (NF1) is an autosomal dominant disorder characterized by the development of nerve sheath tumors, particularly neurofibromas. Specific criteria have been established to facilitate the diagnosis of NF1.

Participants in this study were chosen from patients assessed in the NF Clinic at MGH. The criteria for inclusion in the study were multiple pathologically proven neurofibromas and the lack of other stigmata of NF1. Eight patients were selected for inclusion in this study and were administered a standardized questionnaire. They underwent a clinical examination, including Wood's lamp inspection and neurologic and ophthalmologic evaluation with slit lamp examination. Archived pathologic material from 18 tumors previously resected from 7 of 8 patients and 2 tumors from affected family members were reviewed.

Mean age of onset of symptoms in these patients was 20.3 years. Presentation was varied and included pain, neurologic change and painless masses of the trunk and extremities. Tumor load was extensive in 5 patients and involved the paraspinal regions, brachial plexis, extremities and pelvis. Three patients had more localized tumor distribution. Family history was positive and consistant with autosomal dominant transmission in only 1 of 8 patients. Major morbidity was pain and deformity due to increased tumor number and size. Examination revealed readily palpable, firm, nodular, subcutaneous tumors. Tumor pathology was consistent with neurofibroma or plexiform neurofibroma.

NF1 is often diagnosed in childhood due to the presence of cutaneous/intracranial tumors, CAL spots and Lisch nodules. We report a series of adult patients with isolated multiple neurofibromas who do not meet the clinical criteria for NF1. Our findings suggest that these individuals may comprise a distinct form of NF. Studies are underway to further define the clinical spectrum and molecular basis for this rare disorder.

Program Nr: 1878 from the 1999 ASHG Annual Meeting

Frequency of celiac disease in Down syndrome in the United States. *J. Mackey, G. Worley, W. Treem, A. Boney, P. Hart, L. Stanford, P. Kishnani.* Pediatrics, Duke University, Durham, NC.

The increased prevalence of immune-related disorders in children with Down syndrome (DS) is well recognized. Results of epidemiologic studies in Europe suggest an association between celiac disease (CD) and DS. The frequency of CD in DS is reported to be 1% -17% in Europe, as compared to an incidence of 1:3,000-10,000 live births in the pediatric population in the United States (US). The frequency of CD in DS in the US has not been reported. Because we expected underdiagnosis of CD in children with DS, we developed a clinical algorithm for screening in our DS clinic population. All patients > one year of age, with gluten in their diet and with a confirmed karyotypic diagnosis of DS were evaluated for CD through clinical and laboratory evaluations [IgG anti-gliadin antibody (IgGAGA), anti-endomysial antibody (EMA), IgA anti-gliadin antibody (IgAAGA)]. Children with symptoms, an AGIgG > 2 times reported normal, a positive EMA, or positive IgAAGA were referred for a small bowel biopsy. In cases where IgGAGA was elevated with a normal IgAAGA, an immune profile was performed to exclude possible selective IgA deficiency. 92 children [57M: 35F age range 1-22 years (mean 5.8 years)] have thus far been screened according to the algorithm. Of the 25 children who met criteria, 16 underwent small bowel biopsy (9 lost to follow up or refused). Villous atrophy, reversed crypt to villous ratio and intraepithelial lymphocytes consistent with CD, was found in 3 patients ages 3, 6 and 10 (2M, 1F) giving a frequency of CD in DS in our population of 3.2%. All three with biopsy proven CD also had a positive EMA; in contrast, only 1 /13 children with a normal biopsy had a positive EMA (sensitivity 100%, specificity 90%). None has thyroid disease or diabetes mellitus. Currently all 3 are on a gluten-restricted diet; serologic tests have not been repeated in any thus far. This prospective study supports the view that there is increased prevalence of CD in children with DS also in the US. We thus recommend screening for CD in children with DS older than one year of age who have been exposed to gluten in their diet. A larger study of this issue is warranted.

Program Nr: 1879 from the 1999 ASHG Annual Meeting

Factor V Rsa I polymorphism as a possible prognosticator of certain complications in Sickle Cell Disease. *R.B. Markowitz[1,2], A. Kutlar[1]*. 1) Dept. of Medicine, Sickle Cell Center; 2) Inst Molecular Medicine & Gen, Medical Col Georgia, Augusta, GA.

The underlying cause of Sickle Cell Disease (SCD) is a single nucleotide mutation in the 6th codon of the human b-globin gene, resulting in a Glu to Val substitution. Its primary effect is the polymerization of deoxy Hb S, which leads to chronic hemolytic anemia, microvascular obstruction, and organ damage. Sickle Cell Disease is clinically heterogeneous; the frequency and severity of vaso-occlusive crises vary among individuals. Other serious complications, e.g., cerebrovascular accidents, acute chest syndrome, avascular necrosis and venous thrombosis, occur only in certain patients. Multiple genetic influences are thought to play a role in determining the occurrence of these complications in affected individuals. Our laboratory is interested in identifying genetic factors that might be associated with certain complications in SCD. We tested for the presence of mutations known to be associated with thrombophilia in a cohort of patients with a history of stroke, avascular necrosis, or severe venous thrombosis. The Factor V Leiden and Prothrombin 20210 mutations were virtually absent in patients with and without these complications. However, the Rsa I polymorphism in the Factor V gene is present. Nearly 20% of all SCD patients tested were heterozygous. The allele frequency for R3 was 0.087 in patients with the complications, and 0.1125 in those without. The allele frequency for R2 was 0.032 in the thrombotic group, but R2 was absent from the non-thrombotic group. Less than 5% of African-Americans without SCD showed these polymorphisms. These preliminary data suggest that the Rsa I polymorphisms are not rare among African-Americans and SCD patients. In addition, R2 may serve as a prognosticator for these complications in SCD. The R2 allele is known to be associated with resistance to activated protein C (APC), which is commonly associated with venous thrombosis. We are using a functional assay to determine if SCD patients with R2 and R3 display APC resistance. Larger numbers of SCD patients with and without these complications are being studied for the Rsa I polymorphisms.

Program Nr: 1880 from the 1999 ASHG Annual Meeting

Aplasia cutis congenita and sagittal sinus anomaly. *D. Massocco[1], C. Bellini[1], P. Mezzano[1], M. Lituania[2], P. Fondelli[3], E. Zullino[1], F. Campone[1], W. Bonacci[1], G. Serra[1].* 1) Servizio di Patologia Neonatale, Universita Genova, Istituto Gaslini, Genova, Italia; 2) Divisione di Ostetricia e Ginecologia, Istituto Gaslini, Genova, Italia; 3) Servizio di Neuroradiologia, Istituto Gaslini, Genova, Italia.

Aplasia cutis congenita (ACC; MIM107600) is a congenital absence of the skin.The scalp is the principal site of the involvement and the defect often involves the calvaria and the dura madre. The defect may be part of malfomative syndromes. The aetiology remains obscure. Case report: a female infant was born at 38 week gestation to healthy non-consanguineous parents. No maternal abuse of alchool, smoke or drugs neither intrauterine infection were reported. Amniotic fluid alphafetoprotein was normal. APGAR was 8/9. Birth weight was 2580 g, lenght 45 cm, head circumference 32 cm (all at 25-50 centile). At birth a cutaneous necrotic area near the bregmatic fontanelle was observed. Horizontalization of ocular edge, hypertelorism, low set ears, short nasal filtrum, short neck with pterigium, broad and short hand fingers, bilateral proximal implantation of thumb were present. Karyotype was 46, XX. Skull radiographs evidenced poor and irregular ossification of frontal edges and of parietal bones near to the sagittal suture. Ultrasonic study was normal. CT-scan showed irregular frontal profiles, aspecific calcification of the frontal white matter, normal ventricles and subaracnoideal spaces. Angiographic-MR study evidenced bifid sagittal sinus. Fundus oculi was normal. Hand radiographs excluded morphological and structural anomalies, renal and cardiac ultrasound were normal, karyotipe was normal so far excluding the principal abnormalities (1). Multiple theories have been advanced to explain the aetiology of ACC. A case of ACC with communication of the patent sagittal sinus and the superficial dilated scalp veins have been reported (2). More recently ACC was described in association with high-flow sinus pericranii (3). Our case, associated with the bifid sagittal anomaly, adds further value to the vascular aetiologic hypotesis. 1)Neuroradiology 36:480,1994. 2) Pediatr Neurosurg 17:203,1992. 3) Pediatr Neurosurg 28:79,1998.

Program Nr: 1881 from the 1999 ASHG Annual Meeting

Trisomy 9: A comparison of mosaic Trisomy 9, duplications of chromosome 9 and Trisomy 9 derived from unbalanced translocations. *G.L. Matika[1], C.A. Bay[1,2], M.A. Del Vecchio[1], S. Wright[1]*. 1) Division of Medical Genetics, Children's Hosp of Pittsburgh, Pittsburgh, PA; 2) University of Pittsburgh School of Medicine, Pittsburgh, PA.

In cooperation with the support organization for Trisomy 9, we designed a survey to further elucidate the phenotype associated with Trisomy 9. One goal of the study was to compare the outcome of individuals with mosaic Trisomy 9 (Group A; n=7), duplications of chromosome 9 (Group B; n=8) and individuals with unbalanced translocations involving chromosome 9 (Group C; n=14). Variations existed within each of the groups based on the extent of trisomy present for chromosome 9 and whether there was imbalance for an additional chromosome. Based on the results of the survey, we found that pregnancy complications such as spotting and certain perinatal complications including RDS and jaundice were more common in Group C. Decreased fetal movement, prenatal ultrasound abnormalities, decreased growth parameters at birth and poor Apgar scores were more common in Group A and equally as common in Groups B and C. Although all individuals had pediatric difficulties, microcephaly, failure to thrive, seizures, feeding difficulties, ophthalmologic, and orthopedic problems were more common amongst individuals in Group C. Cardiac abnormalities were more common in Group A and hypotonia was more common in Group B. GI, GU and behavior problems were equally common among the three groups. Overall, developmental delays were more severe in Group A, however the one individual with the most age appropriate development was in Group A. Gross motor skills including rolling, sitting, and walking were attained at the earliest age in Group B. Although information regarding language skills was limited, the average age of the onset of babbling and first word was closer to age appropriate in Group C. In conclusion, there are differences in phenotypic expression between the three Groups, based on their karyotypic abnormality. In addition, our data is also consistent with the findings of Wilson et al, 1985 which documented that the larger the extent of the trisomy for chromosome 9, the more severe and variable the phenotype.

Program Nr: 1882 from the 1999 ASHG Annual Meeting

Multiple etiologies for hemifacial microsomia. *A.M. Mc Inerney[1], D. Donnai[2], M. Calvert[3], M. Bitner-Glindzicz[4]*. 1) NHGRI, NIH, Bethesda, MD; 2) MGB, St. Mary's Hospital, Manchester, UK; 3) Dental Dept, Great Ormond Street Hospital, London, UK; 4) MGB, Institute of Child Health, London, UK.

Hemifacial microsomia (HFM) results from underdevelopment of one or more branchial arches. This typically causes facial asymmetry and conductive hearing loss, usually due to narrowing of the external auditory canal. Most patients have preauricular skin tags and many also have malformed ears (microtia or anotia).

Due to the asymmetric nature of HFM, it is widely regarded as a non-genetic condition. However, familial cases with autosomal dominant inheritance have been observed[1]. An empiric recurrence risk of 2-3% is usually given to parents of an affected child in the absence of a positive family history. One study showed a 10% recurrence risk in first and second-degree relatives of affected individuals[2]. No other studies have confirmed or contradicted these results.

We reviewed the family and antenatal histories of 32 probands for the spectrum of abnormalities associated with HFM. 3 of 32 individuals (9.4%) had a positive family history, suggesting autosomal dominant inheritance with reduced penetrance.

There was a 3-fold increased incidence of twinning (3 sets in 32 cases) with two MZ and one DZ pairs. All three sets were discordant for HFM and negative for family history. This supports previous findings of a vascular origin for some cases of HFM[3]. The DZ pair may suggest a compressive etiology as well.

The HFM phenotype may result from two different mechanisms. The recurrence risk in the presence of a positive family history approaches 50%. Many others may have an environmental etiology, with a recurrence risk much less than than the empiric 2-3%.

1) Mc Inerney A, Winter RM and Bitner-Glindzicz M 1998, Developments in Genetic Hearing Impairment, Whurr Publishers Ltd, p136-140

2) Rollnick BR et. al, Am J Med Gen 26:361-375, 1987

3) Poswillo D, J Maxillofac Surg 2:64, 1974.

Program Nr: 1883 from the 1999 ASHG Annual Meeting

PHACE syndrome: an association of facial hemangiomas and congenital malformations. *C. McKenna[1], L. Krystal[2], R. Shenoy[3], S. Levine[4], D. Kholwadwala[3], L. Mehta[1].* 1) Medical Genetics; 2) Pediatric Dermatology; 3) Cardiology; 4) Ophthalmology. North Shore Univ. Hospital-N.Y.U School of Medicine, Manhasset, N.Y.

There are at least 43 reviewed cases of a constellation of anomalies represented by the acronym PHACE: Posterior fossa malformations, Hemangiomas, Arterial anomalies, Coarctation of aorta and Cardiac defects, and Eye abnormalities (Frieden, 1996). Ventral wall anomalies such as sternal clefts are also reported. While Dandy-Walker malformation and hemangiomas have been reported to co-exist, the extended spectrum of anomalies listed above is rarely recognized. We report a female infant with features of this association. She was the full term product of an uncomplicated pregnancy. Birth weight was 4010 gm. and no facial dysmorphic features were present. A double outlet right ventricle with V.S.D was diagnosed; head sonogram showed Dandy Walker malformation with no hydrocephalus. A quadrantic lens opacity with adherent leukoma was present in the right eye. High resolution blood chromosomes and FISH testing for a 22q11.2 microdeletion were normal. At 4 weeks age, progressively increasing bluish, tense swellings appeared on the left side of the face, identified as dermal to subcutaneous hemangiomas that eventually involved the entire left cheek, temporal and periorbital regions. About 4-5 small cafe-au-lait spots were present over limbs and trunk. The hemangiomas regressed dramatically with high-dose prednisone but recurred and still present a significant concern at age 1 year. The infant is stable from cardiac and neurological aspects. As reviewed by Frieden, most reported cases of this association are female (88%) and all were identified through large, plaque-like facial hemangiomas, unlike those in our patient. The hemangiomas may be aggressive and can involve airways; posterior fossa malformations are present in 74%, arterial anomalies in 41%, cardiac or aortic anomalies in 26% and eye anomalies in 23%. This combination of birth defects suggests an early prenatal developmental field defect. Awareness of these defects is important in the evaluation and management of these infants.

Program Nr: 1884 from the 1999 ASHG Annual Meeting

Multiple schwannomas in identical twins. *W.C. McKinnon[1], A.E. Guttmacher[2], L.B. Jacoby[3], M. MacCollin[4]*. 1) Dept Pediatrics, Univ Vermont Col Medicine, Burlington, VT; 2) National Human Gemone Research Institute, National Institutes of Health, Bethesda, MD; 3) Molecular Neurogenetics Unit, Massachusetts General Hospital, Charlestown, MA; 4) Neuroscience Center, Massachusetts General Hospital, Charlestown, MA.

Schwannomatosis is a rare condition characterized by multiple nonvestibular schwannomas in the absence of other clinical signs of neurofibromatosis type 2 (NF2). Tumors from patients with schwannomatosis frequently harbor NF2 gene mutations and loss of heterozygosity, however, germline NF2 mutations are not found. Some individuals with multiple schwannomas are somatic mosaics for an NF2 mutation, while others, particularly those with a family history, appear to have an inherited predisposition to form tumors that carry somatic alterations of the NF2 gene. We present a case of identical twins with multiple schwannomas and the molecular analysis on a tumor from one of the twins. The proband has multiple skin tumors, which first appeared on the legs between the ages of 10 and 12, and later on the arms, abdomen, and back. At age 26, the proband had a thoracic nerve root tumor removed and pathological analysis revealed a schwannoma. Detailed dermatologic, ophthalmologic, and radiologic examinations showed no evidence of NF1 or NF2. The probands identical twin had an eosinophilic granuloma removed from his skull as a child. Skin tumors appeared in the second decade in a pattern similar to his twin. MRI of his spine showed no lesions. The family history is of note for a sister who had a pilomatricima removed from her arm. No other family member has a pathological verified tumor and the sister, mother, and maternal grandmother have been examined in detail with no evidence of NF1 or NF2. The family is of French Canadian ancestry. Tumor analysis from the proband's spinal schwannoma revealed a truncating mutation in the NF2 gene in exon 14 (1549 ins 29 base pairs). Schwannomatosis is a rare, third major form of NF. To our knowledge, this is the first report of identical twins concordant for the disorder. Further studies are underway to define the extent of the disease and the underlying molecular basis in this family.

Program Nr: 1885 from the 1999 ASHG Annual Meeting

Haas (Type IV) Polysyndactyly Presenting Prenatally with Bilateral Tibial Aplasia. *E.W. McPherson[1,2], W.A. Hogge[1,3]*. 1) Dept Genetics, Magee Womens Hosp, Pittsburgh, PA; 2) Dept Human Genetics, Univ of Pittsburgh, GSPH, Pittsburgh, PA; 3) Dept of ObGyn, Univ of Pittsburgh, School of Medicine.

A 34 yo primigravida presented at 20 weeks because of fetal limb anomalies discovered on routine ultrasound. The fetus was appropriate for gestational age and the anomalies, which were confined to the limbs, included polydactylous severely clubbed feet, bilateral tibial aplasia, bilateral mild radial hypoplasia and cupped hands. The father had a history of bilateral cutaneous syndactyly of the third, fourth and fifth fingers with polyphalangism and polydactyly of the left thumb. Following surgery his hand function was excellent. His feet were unaffected. The fetal karyotype was normal. Due to the severity of the limb defects, the couple decided to terminate the pregnancy.

The 350 gr fetus had severe limb anomalies but no facial dysmorphism and no internal malformations. The wrists were in mild radial deviation. The hands were cupped with complete cutaneous syndactyly of all digits and fusion of the nails. There were six digits including a duplicated hypoplastic thumb on the right and five digits but no recognizable thumb on the left. There were bilateral severe equinovarus feet due to tibial aplasia. Both feet showed polysyndactyly with fusion of the nails.

The fetal hands and feet were typical for Haas (type IV) polysyndactyly which is an autosomal dominant with marked intrafamilial variability. Several typically affected patients have had a parent with partial syndactyly similar to the father of our patient. One previously reported patient had unilateral tibial aplasia. Because the frequency of Haas polysyndactyly is estimated at 1/300,000 and that for tibial aplasia is 1/1,000,000 coincidental occurrence of these anomalies in a second patient is extremely unlikely. Despite thumb involvement, radial hypoplasia has not been previously reported in Haas polysyndactyly. Our case extends the phenotypic spectrum of Haas polysyndactyly to include bilateral tibial aplasia and radial hypoplasia.

Program Nr: 1886 from the 1999 ASHG Annual Meeting

Pallister Hall syndrome:early diagnosis and natural history. *N.J. Mendelsohn[1,2], C. Ludowese[1,2], R. Kriel[1,2], O.A. Schirripa[3], D.K. Manchester[3], L.G. Biesecker[4].* 1) Dept Pediatrics/Med Genetics, Hennepin County Medical Center, Minneapolis, MN; 2) University of Minnesota, Minneapolis, MN; 3) University of Colorado Health Sciences Center; 4) GDRB, NIH/NHGRI, Bethesda, MD.

Pallister Hall Syndrome(PHS) is a condition with hypothalmic hamartoma(HH), polydactyly, bifid epiglottis and visceral anomalies. Hall et al first delineated the syndrome in 1980. Multiple case reports have broadened the diagnostic features from a lethal presentation to one where multiple generations are affected in an autosomal dominant manner. We report two infants diagnosed at an early age with a large HH and polysyndactyly. These infants demonstrate the imporance of early recognition and diagnosis as well as the natural history of PHS.

A 1st patient presented at ten weeks of age with polydactyly and feeding difficulties. ENT consultation revealed bifid epiglottis. A cerebral MRI disclosed a large suprasellar mass. Neurosurgical resection was planned but afer extensive discussion and evidence of no change in size of the mass, He was diagnosed with a benign HH as part of the PHS. A 2nd child presented at 16 days of age with polysyndactyly and a UTI. Further evaluation revealed an anterior rectal fistula. MRI of her head disclosed a HH. We present photographs of the children, subsequent MRIs, growth charts and results of endocrinologic evaluations.

These cases demonstrate important clinical aspects of Pallister Hall Syndrome. Polysyndactyly of PHS is typically central or postaxial and careful attention should be paid to families with four limb polydactyly. This in combination with midline defects should prompt a cerebral MRI. The correlation with these children's other congential malformations provided the correct diagnoses and avoided surgical removal of a major portion of their hypothalamic regions. Characteristically of PHS, the tumors have now been followed with no enlargement of the hamartoma and no evidence of malignant transformation. PHS has been mapped to 7p13. Biesecker et al reported two PHS families with frameshift mutations in GLI3. Evaluation of the GLI3 region is in progress for these children.

Program Nr: 1887 from the 1999 ASHG Annual Meeting

Autosomal dominant dilated cardiomyopathy with subclinical skeletal muscle involvement. *L. Mestroni[1], S. Miocic[3], G. Sinagra[4], G.L. Brodsky[1], M.R. Di Barletta[5], A. Mateddu[2], D. Toniolo[5], F. Muntoni[2].* 1) Dept Molec Genetics, CV-CVI, Univ Colorado, Aurora, CO; 2) Dept. of Paediatrics, Imperial College, London U.K; 3) ICGEB, Trieste, Italy; 4) Dept. of Cardiology, Ospedale Maggiore, Trieste, Italy; 5) IGBE-CNR, Pavia, Italy.

Background: Dilated cardiomyopathy (DC) is a heart muscle disease, characterised by impaired myocardial contractility and dilatation of the left or both ventricles, which is the leading indication for heart transplantation. DC is frequently inherited (familial DC or FDC) and genetically heterogeneous. The aim of this work was the study of a subgroup of FDC families characterized by autosomal dominant transmission and subclinical skeletal muscle disease. **Methods**: Relatives of 40 index patients with FDC underwent a detailed pedigree reconstruction and a non-invasive clinical screening with electrocardiography and echocardiography. Affected relatives completed the investigations with cardiac catheterization and endomyocardial biopsy. An accurate neurological examination including a skeletal muscle biopsy (SMB) was performed whenever possible. **Results**: Out of 5 families with FDC showing subclinical signs of skeletal muscle involvement, three families presented an autosomal pattern of inheritance, therefore excluding X-linked DC. In family MDDC2 and MDDC3 the cardiac disease was characterised by arrhythmia, severe left ventricular dilatation and dysfunction, segmental wall motion abnormality, and significant improvement with medical treatment. The neurological examination was unremarkable. Family MDDC1 was characterized by the presence of histological myocarditis and highly variabile skeletal muscle involvement in the affected individuals, from none to mild signs of limb-girdle muscle distrophy, or mild rigidity of the elbows, spine and Achilles tendon. Serum CK levels were variable from normal to slightly increased even in the same individual. SMB and EMG were invariably pathological. **Conclusions**: Skeletal muscle disease can underlie FDC and must be carefully investigated. Genes expressed in both skeletal and cardiac muscle may be candidates for causing this disorder.

Program Nr: 1888 from the 1999 ASHG Annual Meeting

Congenital intracranial lipoma. *P. Mezzano, C. Bellini, D. Massocco, E. Zullino, F. Campone, M. Mazzella, W. Bonacci, G. Serra.* Servizio di Patologia Neonatale, Universita Genova, Istituto Gaslini, Genova, Italia.

Case report: a male infant was born by vaginal delivery at 34 weeks of gestational age of uneventful pregnancy (weight 2025 g, lenght 45 cm, head circumference 32 cm; all parameters at 25-50 percentile). Physical evaluation showed multiple ecchymoses (face and left arm) and back dermal sinus on the midline. Shortly after birth he developed respiratory distress syndrome that needed ventilatory support. Neurological examination revealed clonus of the extremities. A routinary cerebral echographic evaluation revealed an hyperechoic image (0.5x0.3 cm, median sagital scan) situated down to the splenium of the corpus callosum, first referred to a possible hemorrage. CT images revealed hypodense lesion that did not enhance after contrast injection. T1-weight images demonstrated marked hyperintense lesion of the velum interpositum region. These radiological findings strongly supported the diagnosis of intracranial lipoma (ICL). ICLs are uncommon, benign and congenital malformations of the central nervous system, most often found in the midline, whose development remains poorly understood. Incidence is estimated at less than 0.1% of all intracranial tumors. The most common sites of occurrence are corpus callosum, quadrigeminal/superior cerebellar cistern, suprasellar/interpeduncolar cistern, cerebello-pontine angle cistern, and sylvian cistern. At least half of these lipomas are associated with other brain anomalies. Generally they are asymptomatic, becoming symptomatic because of both the site of lesion and of the involvment of the surroindings structures in regressive change within the lipoma tissue. ICLs formation might result from abnormal persistence and maldifferentiation of the meninx primitiva into lipomatous elements, so considering ICL as a choristoma.

Program Nr: 1889 from the 1999 ASHG Annual Meeting

Autism: family histories concordant for alcoholism. *J.H. Miles, N. Takahashi, C. Jones, R.E. Hillman.* Univ. Missouri Hospital and Clinics, Columbia, MO.

Genetic overlap between autism and other neuropsychiatric disorders is suggested by 1) reports that alcoholism, affective disorders and schizophrenia aggregate in families ascertained through an autistic child, 2) overlap of candidate genes/areas identified for neuropsychiatric disorders (AJMG.79.1998), & 3) biological/pharmacological evidence that dysregulation of the neurotransmitter systems (serotonin, dopamine, etc) are involved in all these disorders. To begin to define the neuropsychiatric disorders which segregate together, families ascertained through an autism proband were studied to determine prevalence and pedigree configuration of associated neuropsychiatric disorders. We suspect subsets of autism families with similar family histories will be more homogeneous. A consecutive sample of 119 autism probands with complete pedigrees were analyzed. Family history was ascertained using direct semi-structured interviews (family history method). A significant family history of alcoholism (i.e. an affected 1^0 relative; 2^0 relative plus 3 others in a mendelian pattern; or at least 4 affected relatives in the same family branch) was observed in 34% (41/119) of the families. **Results:** In hi-alcohol families, 20% mothers, 61% fathers, 15% MGM, 44% MGF, 25% PGM, 42% PGF were alcoholics. The hi-alcohol autism families had more relatives with an autistic disorder (34% vs. 15%, $p<0.02$), and affective disorders (49% vs. 18%, $p<0.001$), and a higher sibling recurrence (17% vs. 10%, $p<0.2$). These findings were the same for unilineal and bilineal (for alcohol) families and not influenced by the sex of the transmitting parent. In hi-alcohol families, probands had significantly higher IQs/DQs ($p<0.05$), were less likely to be macrocephalic, and more likely to have regressed at onset; families were of lower SES. Hi and low alcohol families reported no difference in cognitive or language disorders, dyslexia, ADHD or seizures. The high incidence of alcoholism in autism families and different features in hi vs. low alcohol families indicates there are genes which predispose to both autism and alcoholism. The lack of association between alcoholism and autism in the alcohol literature suggests that only a subset of alcoholism is caused by these genes.

Program Nr: 1890 from the 1999 ASHG Annual Meeting

Dysmorphic facial features, CNS abnormalities, hand malformations, and myocardial cytomegaly in three female fetuses: A familial fetal neurodegenerative disorder? *P.T. Mohide[1,2], M.J. Huggins[1], J.A. Ramsay[1,2,3], J. Woulfe[1,2,3], J. Provias[1,3], K. Chorneyko[1,2,3], M.J.M. Nowaczyk[1,2,3].* 1) Faculty of Health Sciences, McMaster Univeristy, Hamilton, Canada; 2) Hamilton Health Sciences Corporation, Hamilton, Canada; 3) Hamilton Regional Laboratory Medicine Program, Hamilton, Canada.

A 36 year old G4TA2E1 woman with bilateral fifth finger clinodactyly, minimal cutaneous toe syndactyly, and didelphic uterus had three female fetuses with a unique association of malformations. Her mother and brother had polycystic kidney disease, her renal ultrasound (US) was normal. She was referred at 22 weeks gestation in her first pregnancy for evaluation of abnormal brain contour identified by US. The pregnancy was terminated and postmortem examination showed a dysmorphic fetus with bitemporal narrowing, fifth finger clinodactyly, low set ears, retromicrognathia, 11 pairs of ribs, bilateral club feet, and a thickened frenulum bisecting the upper alveolar ridge. Neuropathologic abnormalites included agenesis of the olfactory bulb and of corpus callosum, minimal frontal lobe hemispheric fusion,and marked midline fusion of the deep gray matter nuclei. In the third and fourth pregnancies, US examinations were normal until 19 and 13 weeks gestation, but repeat US three and two weeks later respectively showed similar cranial abnormalities with overlapping skull bones. External dysmorphic features were similar in the three fetuses. Myocardial cytomegaly was demonstrated microscopically in the absence of congenital heart disease, infection, or cytomegaly in other organs. Ultrastructural studies showed accumulated mitochondria in the enlarged myocardiocytes. The following were normal: electron transport chain assays and lactate/pyruvate ratio in culured fetal skin cells, and amniotic fluid organic acid and 7-DHC levels. The fetal karyotypes were 46,XX. To our knowledge this pattern of malformations has not been reported previously. It is possible that the CNS anomalies are a result of a fetal neurodegenerative disorder progressing during the second trimester, however, at the present time the cause of these abnormalites remains unknown.

Program Nr: 1891 from the 1999 ASHG Annual Meeting

NF1 and overgrowth syndrome resembling Weaver syndrome in 3 sporadic patients. *A. Moncla[1], C. Missirian[2], O. Pincemaille[3], J. Mancini[2], M.O. Livet[2], P. Malzac[1], N. Philip[1].* 1) Department of Medical Genetics, Hopital d'Enfants de la Timone, Marseille, France; 2) Department of Neuropediatrics, Hopital d'Enfants de la Timone, Marseille, France; 3) Department of Pediatrics, Bastia, France.

We report three unrelated patients presenting the combination of an overgrowth syndrome resembling Weaver syndrome and NF1. This association has been described by Hennekam et al (J Med Genet, 1998)in two related patients. The three patients were referred for unexplained mental retardation with behavioral problems. All of them exhibited a remarkable coarse face with hypertelorism, epicanthic folds, large ears, fetal finger pads and hyperextensible fingers. Height and head circumference were above the 97th centile and osseous maturation was advanced in all. All presented more than 5 cafe-au-lait spots. One of them developed precocious puberty related to intracranial glioma. Moderate psychomotor and language delay were present with a particular behavioral phenotype. All three patients had a hoarse and low-pitched voice. RHG-banded karyotypes were normal. Molecular analyses of the 17q11.2 region are in progress to test the hypothesis of a deletion encompassing the NF1 gene.

Program Nr: 1892 from the 1999 ASHG Annual Meeting

Recurrence of Kabuki Make Up Syndrome in siblings. *A. Monier[1], C. Helsemans[2], L. Van Maldergem[1]*. 1) Centre de Genetique Humaine, Inst. Pathologie et Genetique, Loverval, Belgium; 2) Department of Paediatrics, Centre de Sante des Fagnes, Chimay, Belgium.

The Kabuki make-up syndrome is characterized by mild to moderate mental retardation, short stature, prominent fingerpads and characteristic facial features that include long and thick eyelashes, eversion of lateral lower lid, arched eyebrow with sparse lateral third, broad nasal tip, prominent ear lobes and cleft or high arched palate. More than 70 cases have been reported since its first description in 1981 by Niikawa and Kuroki. It usually occurs sporadically, however, in five instances, a possible dominant transmission has been reported. We describe here a boy born to non consanguineous Belgian parents evaluated at 11 years of age for mild mental retardation (IQ 73) , bilateral cleft lip and palate and facial dysmorphia highly suggestive of Kabuki syndrome. His stature was at 50th centile. He underwent surgery for bilateral cryptorchidism. Interestingly, his younger brother also had mild mental retardation (IQ 63) , ventricular septal defect and a facial dysmorphia compatible with Kabuki syndrome. Bilateral renal cortical cysts and left cryptorchidism were also observed. A photograph of the deceased father was normal. He had a digitalized left thumb without any other clinical abnormality. The mother and her three subsequent children are also normal. This is the first report of Kabuki syndrome recurrence in siblings born to normal parents. It may point to gonadal mosaicism in one parent or, alternatively, to genetic heterogeneity of the condition with autosomal recessive or X-linked recessive patterns of inheritance coexisting with an autosomal dominant form.

Program Nr: 1893 from the 1999 ASHG Annual Meeting

The wrinkly skin syndrome: Report of a new case and expansion of the phenotype. *A. Moon, A. Shanske.* Dept. of Pediatrics, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY.

In 1973, Gazit described a disorder in sibs, wrinkly skin syndrome (WSS), characterized at birth by wrinkly skin of the hands and feet who developed hypotonia and winging of the scapulas. Only 9 additional cases have been since reported and most have had prominent neurologic involvement including mental retardation, microcephaly and seizures. We have identified a new case with previously unreported genitourinary and gastroenterologic abnormalities.

PW was the 3.13 kg product od a term pregnancy complicated by polyhydramnios and hydronephrosis diagnosed prenatally. The family history was unremarkable and the parents were unrelated. The newborn exam revealed generalized hypotonia and wrinkled skin over the trunk, abdomen and extremities with sparing of the face and anemia. Ultrasound studies revealed bilateral hydroureters/nephrosis with no refux. Because of poor suck and GER, he required a gastrostomy at 3 months and was hospitalized several times thereafter with pseudoobstruction. Radiologic studies have repeatedly shown multiple dilated loops of small bowel. A skin biopsy showed normal morphology and a normal elastin fiber stain. At 13 months of age he appeared a stigmatized small hypotonic child. His height wsa 68.5 cm(<<3%), his weight was 6.4 kg (<<3%), and his HC was 46.5 cm (25%). He had a preauricular fistula, a tongue-thrust habit, hypertelorism, alternating esotropia and conjunctival melanosis. The skin wrinkling was restricted to the abdomen and the dorsum of the hands and feet and he had a large ventral hernia.

The WSS is a rare connective tissue disorder with prominent neurologic features with no known biochemical defect of collagen. It has been postulated that the pathogenesis of the wrinkled skin is fetal lymphedema and an overabundance of skin. We suggest that perhaps some of the wrinkling is due to urinary tract and gastrointestinal obstruction as observed in our patient. We feel that hydroureter/nephrosis and pseudoobstruction are manifestations of WSS and part of a generalized connective tissue disorder.

Program Nr: 1894 from the 1999 ASHG Annual Meeting

Charcot-Marie-Tooth neuropathy and Duchenne muscular dystrophy segregating in the same family: clinical and genetic studies. *L. Mota-Vieira[1], F. Martins[1], E. Vieira[2], S. Tardieu[3], A. Guimaraes[4], M. Melo Pires[4], E. Medeiros[5], J. Lopes[1], M.R. Santos[2], E. LeGuern[3], J. Santos[1]*. 1) Hosp Ponta Delgada, Ponta Delgada, Portugal; 2) Inst Genetica Medica, Porto, Portugal; 3) INSERM U-289, Paris, France; 4) Hosp St Antonio, Porto, Portugal; 5) Hosp Egas Moniz, Lisboa, Portugal.

In the Portuguese Azorean Islands we identified a family showing segregation of two neuromuscular diseases: Charcot-Marie-Tooth (CMT) neuropathy and Duchenne muscular dystrophy (DMD). In this family the mother, 1 son and 1 nephew are affected with CMT, whereas 2 other sons had a compound phenotype of both diseases. We performed a clinical study, including neurological examination, serum CPK determination, electrophysiological study and nerve and muscle biopsies, and the molecular genetic analyses for both diseases. The two CMT-DMD brothers have generalized weakness, muscle wasting and areflexia, pes cavus and scoliosis, predominantly. The clinical data and the inheritance mode of the hereditary neuropathy were compatible with a CMT1 or a CMTX1. Molecular study showed that the CMT phenotype cosegregates with 17p11.2 duplication. In the CMT-DMD brothers immunohistochemistry analysis showed an abnormal location of dystrophin on the majority of muscle fibers. No deletion or duplication was found on analysis of the DMD gene. Genotyping revelead that the DMD haplotype was of grandpaternal origin, and elevated CPK levels in the mother corroborated her carrier status at the somatic level. One maternal aunt was found to be a non-carrier, based on the haplotype of her son affected with CMT, whereas the other maternal aunts were attributed the a priori risk of 14% of being carriers due to paternal gonadal mosaicism. Our results indicate that the gene dosage of PMP22 associated with the absence of properly localized dystrophin can explain the compound phenotype of both CMT-DMD brothers. To the best of our knowledge these patients are the first cases described. Moreover, the identification of the different mutated genes segregating in this complex family is essential to propose genetic counselling and prenatal diagnosis for all members of the family.

Program Nr: 1895 from the 1999 ASHG Annual Meeting

High rates of schizophrenia in velo-cardio-facial syndrome. *K.C. Murphy[1,2], L.A. Jones[1], M.J. Owen[1]*. 1) Department of Psychological Medicine, University of Wales College of Medicine, Cardiff, UK; 2) Department of Psychological Medicine, Institute of Psychiatry, King's College, London, UK.

Velo-cardio-facial syndrome (VCFS), a syndrome characterised by distinctive dysmorphology, congenital heart disease and learning disabilities, is associated with small interstitial deletions of chromosome 22q11. In view of previous reports of a high prevalence of psychosis in VCFS, we undertook the present study to characterise the psychiatric phenotype in the largest interviewed series to date of adults with VCFS. We evaluated 50 adults with VCFS using a structured clinical interview (Schedules for Clinical Assessment in Neuropsychiatry or Psychiatric Assessment Schedule for Adults with Developmental Disability if IQ < 50) to establish a DSM-IV diagnosis. The schizophrenia phenotype in individuals with VCFS and schizophrenia was compared to a matched series of individuals with schizophrenia without VCFS (n = 12). The Kings Schizotypy Questionnaire was administered to VCFS individuals (n = 41), their first-degree relatives (n = 68) and a series of unrelated normal controls (n = 316). All VCFS individuals were genotyped for a novel promoter polymorphism in the COMT gene and also for a genetic polymorphism in the COMT gene which results in variations in enzymatic activity. Thirty per cent (n = 15) of individuals had psychotic disorder with 24% (n = 12) fulfilling DSM-IV criteria for schizophrenia. In addition, a further 12% (n = 6) had major depression without psychotic features. The individuals with schizophrenia had fewer negative symptoms and a relatively later age of onset compared to individuals with schizophrenia without VCFS. We found no evidence that either a promoter polymorphism or presence of the low activity allele of the COMT gene was associated with psychosis in our sample of individuals with VCFS. The high prevalence of schizophrenia in this group suggests that chromosome 22q11 might harbour a gene or genes of relevance to the aetiology of schizophrenia in the wider population.

Program Nr: 1896 from the 1999 ASHG Annual Meeting

congenital lymphedema,unilateral choanal atresia,pericardial effusion, high arched palate and preauricular pits: A possible new autosomal recessive syndrome. *a.m nadroo, e. lieber, m. zak, i. bialik, d. menos.* pediatrics, new york methodist hospital, brooklyn, NY.

Congenital lymphedema was described by Milroy as an autosomal dominant condition in 1928. Other congenial abnormalities have been described in association with congenital lymphedema, leading to proposals of autosomal dominant, autosomal recessive and X-linked dominant modes of inheritance. We describe a patient with a familial congenital lymphedema, choanal atresia, pericardial effusion and preauricular pits-a possibly undescribed constellation of features. The proband is a 19-year-old female. She was admitted for cellulitis of her left hand. Physical examination revealed lymphedema of lower extremities, both hands and forearms. The patient had preauricular pits and a high arched palate. No other dysmorphic features were noted. She had normal vision and hearing. A review of her records showed that she was a product of a consanguineous marriage and a normal pregnancy, born at 37 weeks of gestation. Her birth weight was 3200g. At the age of one month she had corrective surgery for a left-sided choanal atresia, diagnosed shortly after birth. At the age of 19 months,she developed progressive swelling of both lower extremities, diagnosed as lymphedema. She was admitted for pericardial effusion on three occasions, at the ages of 12, 13 and 14 years. Echocardiograms and renal sonograms failed to reveal any underlying structural cardiac or renal abnormalities. However, the third episode of pericardial effusion was treated with pericardiotomy. The patient has had a normal developmental course and is an average student. Chromosomal studies showed a normal karyotype of 46, XX. There are eight other family members with a variety of phenotypic expressions similar to those present in the patient, all resulting from consanguineous marriages. All the parents are phentypically normal. Three individuals have all five of the phenotypic features present in the patient, including two brothers of the proband.The association of familial lyphedema with unilateral choanal atresia, pericardial effusion, high arched palate and preauricular pits, discloses a possible new autosomal recessive syndrome.

Program Nr: 1897 from the 1999 ASHG Annual Meeting

Deletion 2q37.1 in a child with familial paroxysmal kinesiogenic choreoathetosis. *R. Naeem, A.H. Jackson, G. Cohn, J. Sullivan, H.E. Gilmore.* Departments of Pathology, Pediatrics, Obstetric/Gynecology and Neurology, Bay State Medical Center and Tuft University School of Medicine, Springfield and Boston, MA.

Genes for paroxysmal choreoathetosis have been recently mapped using linkage analysis by two independent groups to chromosome 2q. We present a 7 year old child with familial paroxysmal kinesiogenic choreoathetosis with a deletion in the long arm of chromosome 2 (q37.1). This child presented with mildly delayed motor development but fairly normal language development. At about 17 months of age he developed episodes in which he would have dystonic extensor posturing of the arms which were later noted to be precipitated by activity such as playing with a puzzle. Except for mild cognitive impairment, his neurologic exam was normal. Genetic evaluation revealed evidence of a high arched palate, mild hypotonia and umbilical hernia, but no other significant dysmorphic features. Family history revealed that one of the authors had evaluated the maternal uncle in the past with the same diagnosis. Peripheral karyotypes of mother, affected uncle, maternal aunt and maternal grand parents were normal. Paternal side of history and karyotypes are unavailable. Lymphoblast cell lines from all these family members have been established and further studies will be performed with collaborations. As most of these disorders are probably due to the defects in ion channel genes and there is a cluster of ion channel genes on distal 2q, this case may represent the precise cytogenetic region involved with this disorder and may be helpful for identifying and cloning candidate genes.

Program Nr: 1898 from the 1999 ASHG Annual Meeting

A new X-linked mental retardation syndrome with distal limb defects, hearing impairment, verrucosis and immunodeficiency. *J.C. Oosterwijk[1], A. Wischmeijer[1], M. Losekoot[2], A. Haraldson[3], G. Theunissen[4], I. van Gelderen[5].* 1) Dept Medical Genetics, Groningen Univ, Groningen, Netherlands; 2) Dept Medical Genetics, Leiden Univ Medical Centre, Netherlands; 3) Dept Pediatrics, Univ Hospital, Reykjavik, Iceland; 4) Hooge Burch, Zwammerdam, Netherlands; 5) Eemeroord, Baarn, Netherlands.

We descibe 3 related male patients who are affected with a condition characterized by mild mental retardation, a varying degree of a/symphalangism of hands and/or feet, hearing loss, extensive verrucosis and mild impairment of both cellular and humoral immunity. Additional features are dysplastic ears, scoliosis, hypermetropia, congenital heart defects, body hypertrichosis and facial dysmorphic features. We assume X-linked inheritance in this familial non-progressive retardation syndrome. Two female obligate carriers and one female at 50% risk showed now features other than from mild symphalangism of several toes on extensive investigation. The differential diagnosis of this unusual and previously undescribed combination of symptoms is discussed. DNA analysis using polymorphic markers spanning the entire X-chromosome revealed that the patients share a region from Xp11.4 to Xq24, approximately 90 Mb.

Program Nr: 1899 from the 1999 ASHG Annual Meeting

Growth manifestations and development in the cri-du-chat syndrome. *J. Overhauser[1], M. Kouahou[1], R.C. Marinescu[1], P. Cerruti Mainardi[2].* 1) Dept. of Biochem/Molec Pharm, Thomas Jefferson Univ, Philadelphia, PA; 2) S.Andrea Hospital, Vercelli, Italy.

Cri-du-chat syndrome is a partial aneusomy syndrome that is associated with a deletion of the short arm of chromosome 5. At birth, a cat-like monochromatic cry is heard and is considered diagnostic for the syndrome. In addition, a microcephalic round face with hypertelorism and low birth weight are typically observed. As the infant ages, both growth and developmental delays are common. In order to determine the normal growth pattern for children with cri-du-chat syndrome, growth parameters were obtained for children with cri-du-chat syndrome from ages 0-18. Through the cooperation of members of the US, Australian, and Italian cri-du-chat syndrome parent support groups, pediatric records as well as parent reporting of growth information from over 250 females and 150 males were obtained. Height, weight, and head circumference growth curves have been developed for both males and females and are compared with the growth curves from the normal population. The development of these growth curves will be useful for physicians when determining when nutrient intervention is needed. In addition, information on the age of accomplishment of various developmental milestones was obtained. This information will be useful to parents and physicians in anticipating what and when certain developmental milestones will be achieved by children diagnosed with cri-du-chat syndrome.

Program Nr: 1900 from the 1999 ASHG Annual Meeting

Miller-Dieker syndrome due to ring chromosome 17. *A.P. Pai[1], N. Al-Sanah[2], S. Blaser[3], D. Chitayat[1,2].* 1) The Prenatal Diagnosis Program, The University Health Network, University of Toronto, Toronto, Ontario, Canada; 2) Department of Pediatrics, Division of Clinical Genetics, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada; 3) Department of Pediatrics, Division of Radiology, Hospital for Sick Childen, University of Toronto, Toronto, Ontario, Canada.

Miller-Dieker syndrome consists of lissencephaly, microcephaly, characteristic facies with. vertical furrowing , pre- and postnatal growth retardation, and various other birth defects. About 50-70% of the cases with Miller-Dieker syndrome can be shown to have a deletion of 17p13.3 by light microscopy and almost all the remainder will have a submicroscopic deletion, most easily demonstrated by fluorescent in situ hybridization (FISH). We present a case with ring chromosome 17 and clinical findings consistent with Miller-Dieker syndrome. Prenatally, first trimester ultrasound scanning revealed an increased nuchal translucency measurement of 3 mm. An ultrasound performed at 17 weeks demonstrated mild IUGR with mild polyhydramnios. No other abnormalities were detected. Further ultrasound scans at 30 and 37 weeks showed polyhydramnios, IUGR, query agenesis of the corpus ccallosum and absent cisterna magna. Prenatal chromosome analysis revealed 46,XX,r(17)(p13q q25). Parental karyotypes were normal. MRI showed lissencephaly with an hourglass configuration consistent with Type 1 lissencephaly. A review of the literature has revealed only 3 previous cases where clinical features of Miller-Dieker syndrome were associated with ring chromosome 17. Further studies are being done to delineate the role of dominant genes deleted in this case in the pathogenesis of the clinical findings. This case further emphasize the importance of increased NT in the diagnosis of fetal chromosome abnormalities in general and those associated with fetal akinesia in particular.

Program Nr: 1901 from the 1999 ASHG Annual Meeting

Possible mother-daughter transmission of Wildervanck syndrome. *J.G. Pappas, E. Rimar, V.B. Penchaszadeh.* Div Medical Genetics, Beth Israel Medical Ctr, New York, NY.

The Wildervanck (cervico-oculo-acoustic) syndrome includes congenital perceptive deafness (sensorineural, conductive, or mixed), Klippel-Feil anomaly (fused cervical vertebrae), abducens palsy with retractio bulbi (Duane syndrome), hemifacial microsomia, epibulbar dermoids, facial assymetry, preauricular skin tags and pits, cranial dysostosis and torticollis. Occasional abnormalities include mental retardation, occipital meningocele, pseudopapilledema, hydrocephalus, growth deficiency, cleft palate, ear abnormalities, cardiac defects, cervical ribs, absent kidney and cholelithiases. All reported cases have been sporadic and the disorder is limited to females, raising the question of sex-linked dominance with lethality in the hemizygous male (Wettke-Schafer, R.; Kantner, G.; Hum. Genet. 64: 1-23, 1983). Wildervanck reviewed the subject and concluded that polygenic inheritance with limitation to females is most likely [Handbook of Clinical Neurology. Amsterdam: North Holland (pub.) 32 1978. Pp. 123-130]. Wildervanck syndrome and hemifacial microsomia may be in the same spectrum of developmental disorders (Cohen MM Jr et al, Cleft Palate Journal. 26(4):276-86, 1989). We present a 10 year old girl with Wildervanck syndrome and her mother with hemifacial microsomia. The proband had growth deficiency, asymmetry of face and limbs (right < left), right sensorineural hearing loss, Klippel-Feil sequence, marked scoliosis of the thoracic spine convex to the right, and right sided Duane anomaly, torticollis and Sprengel deformity. The right hand was osteopenic, smaller and delayed in bone maturation. These anomalies are consistent with the diagnosis of Wildervanck syndrome. The chromosome analysis was normal 46,XX. The mother had right microtia, right hearing loss, craniofacial and limb asymmetry (right < left) and history of two first trimester spontaneous abortions. These features are consistent with hemifacial microsomia. The mother of the proband may represent an incomplete presentation of Wildervanck syndrome, lending support to the hypothesis that the inheritance of this syndrome is X-linked dominant with lethality in the hemizygous male.

Program Nr: 1902 from the 1999 ASHG Annual Meeting

A Korean case of partial trisomy 9 by 3:1 segregation of balanced maternal translocation (6q+;9q-). *S.H. Park[1], M.K. Kim[1], Y.T. Kim[2], Y.H. Lee[2], S.H. Park[3], S.H. Lee[4]*. 1) Department of Pediatrics, Korea University, Seoul, Korea; 2) Department of Obstetrics and Gynecology, Korea University, Seoul, Korea; 3) Department of Anatomical Pathology & Institute of Human Genetics, Korea University, Seoul, Korea; 4) Department of Orthopedics, Korea University, Seoul, Korea.

Trisomy 9p syndrome is a disease that the short arm of chromosome 9 is trisomy and was first described by Rethore, et al., in 1970. Almost 100 cases have been reported after that. Clinical manifestations of this syndrome are craniofacial malformation, facial deformity, skeletal and dermatoglyphic abnormalities with variable degrees of mental retardation.

We experienced a case of partial trisomy 9 syndrome in a 15-month-old female who had multiple congenital anomalies of frontal bossing, oblique antimongoloid palpebral fissures, enophthalmos, hypertelorism, globular prominent nose, down-turned mouth, prominent low-set ears, simian creases of both hands, clinodactyly and single crease of 5th fingers, congenital dislocation of both knees and mental retardation. From cytogenetic studies using G banding technique and flourescent in situ hybridization (FISH), she is presented with an extra derivative chromosome 9. The karyotype of the patient was confirmed as 47,XX,+der(9)t(6:9)(q27;q21.2)mat. This will be presented with review of literatures.

Program Nr: 1903 from the 1999 ASHG Annual Meeting

A mild form of autosomal recessive Craniotubular Dyplasia in a large inbred Brazilian family. *M.R. Passos-Bueno[1], W.R. Wilcox[2], N. Alonso[3], L.G. Alonso[4].* 1) Dept Biologia,IBUSP,So Paulo, SP,Brazil; 2) Div.Med.Genet., Cedars-Sinai Med.Cntr., Los Angeles,CA,USA; 3) Dept. Cirurgia Plastica,FMUSP,SP,BR; 4) Dept. Morfologia,UFSP-EPM,SP,BR.

We report a four generation inbred pedigree with 10 patients affected by a mild form of craniotubular dysplasia. The proband is a 3 year old boy born to healthy consanguineous parents. He has normal stature and intelligence, but bony expansion of the nasal root, hypertelorism, and difficulty breathing. Radiographs and 3-D reconstructed computed tomography of the skull showed sclerosis of the skull and hyperostosis of the nasal root, mandible, and maxilla. In the long tubular bones of the limbs there is increased radiodensity of the cortex of the diaphyses with relative lucency of the metaphyses. Epiphyses are normal. Genu valgum was also present His serum calcium was normal. The 9 other affected relatives have similar facial alterations and also no cranial nerve involvement. All affected patients were born to consanguineous and apparently healthy parents. They had a total of 31 healthy descendants. One of the affected individuals, a 46 year old woman, is of short stature secondary to a short neck and scoliosis due to vertebral and rib fusions. Similar vertebral and rib fusions were noted in a 5 year old boy in the family. He is short but does not have any facial dysmorphism. The clinical and radiological abnormalities in the proband are compatible with a mild form of craniometaphyseal dyplasia (CMD). The pedigree in this family indicates an autosomal recessive (AR) inheritance. While most CMD is inherited as an autosomal dominant trait, a rare AR form has been suggested with a more severe phenotype and cranial nerve involvement. Except for one reported case of CMD with a balanced translocation [46, XX, t(12;18)(q13;q12); Surg.Neurol. 27:284-290, 1987], there has been no association of vertebral abnormalities and CMD. The vertebral anomalies in this family may be a rare manifestation of the disorder or may be due to a second, unrelated, disorder. This family confirms the existence of an AR form of CMD and will assist in the mapping and identification of the CMD gene. FAPESP, HHMI, CNPq, PRONEX.

Program Nr: 1904 from the 1999 ASHG Annual Meeting

Familial Beckwith-Wiedemann syndrome (BWS) with t(11;14)(p15.5;q24.2) in three generations. *L.M. Pasztor[1], J. McGrath[2], J. Reese[3]*. 1) Section of Genetics & Molecular Medicine, Children's Mercy Hospitals&Clinics and the U of MO School of Medicine, Kansas City; 2) Department of Comparative Medicine,Yale Univ. Medical Center, New Haven, CT; 3) Department of Pediatrics,Kansas University Medical Center, Kansas City, KS.

Although most BWS cases are sporadic, several genetic etiologies have been identified. Of these, maternally derived balanced translocations and inversions have been among the rarest. We report an inherited balanced translocation in three generations, two of which showed an individual with the BWS phenotype. CLINICAL: The proband, LM, a 3.5 year female, was 4675 grams at birth. Although her mother, LA, a 19 yr. primagravida, carried the clinical diagnosis of BWS from infancy, antenatal testing had not been pursued. LM was LGA in length, weight and head circumference. Her physical exam showed features of BWS, including an enlarged, protuberant tongue and vertical creases on both ears with a small pit in the posterior helix of each pinna. There were no palpable masses and her liver was normal in size. Postnatally, her initial blood glucose level was 42, then fell to 35 and she required IV dextrose infusion. To date, her growth has been normal with no organomegaly or hemihyperplasia. CYTOGENETICS: Because of the clinical diagnosis of BWS in two generations, chromosome studies were performed which revealed a balanced t(11;14)(p15.5;q24.2) in the proband, her mother and unaffected grandmother, LL. LL's siblings had normal karyotypes. FISH studies with whole chromosome paints on metaphase preparations showed chromosome 14 genetic material translocated distally to the short arm of one chromosome 11. However,the reciprocal der(14) could be detected only when an 11p subtelomeric probe was used. SIGNIFICANCE: Although IGF2, a maternally imprinted gene in 11p15.5, is overexpressed in many individuals with BWS, the molecular breakpoints have been centromeric to IGF2 in blanced translocations. It is possible that these breakpoints disrupt a specific gene or remove a cis-acting element. Future molecular dissection of this and other chromosomal rearrangements may reveal the 11p15 loci critical to the pathogenesis of BWS.

Program Nr: 1905 from the 1999 ASHG Annual Meeting

Thyroid agenesis in Peters' anomaly - A new syndrome? *J.E. Pellegrino[1], J.M. Engel[2], S. Shen-Schwarz[3], C. Benito[1], M. Hiatt[4], D. Chavez[1], D. Day-Salvatore[1]*. 1) Department of Obstetrics, Gynecology and Reproductive Sciences, UMDNJ-Robert Wood Johnson Medical School and Saint Peter's University Hospital, New Brunswick, NJ; 2) Department of Surgery, SPUH; 3) Department of Pathology, SPUH; 4) Department of Pediatrics, UMDNJ-RWJMS and SPUH.

Peters' Plus syndrome is an autosomal recessive disorder characterized by Peters' anomaly of the eye, short limb dwarfism, cleft lip or palate, and characteristic facial features. Heart defect, renal anomalies and SUA have been reported. Jung et al. reported two patients with anterior cleavage disorders, short stature, and typical facial features of Peters' Plus with additional features of congenital hypothyroidism, cerebellar hypoplasia, vertebral anomalies, and tracheostenosis. We report a patient with congenital hypothyroidism, cerebellar hypoplasia, hemivertebrae and features of Peters' Plus syndrome. Prenatal ultrasound revealed a single umbilical artery, hemivertebrae, and complex cardiac defect (ASD, VSD, and Truncus Arteriosus). Amnio demonstrated a 46, XY karyotype and normal 22q11 deletion studies. The infant was delivered at 30-weeks-gestation; physical exam was notable for a two-vessel cord, hypertelorism, cloudy corneas, small posteriorly-rotated ears with overfolded helices, flattened nasal bridge, wide-spaced nipples, and fifth finger clinodactyly. Neonatal course was complicated by RDS requiring ventilation, congestive heart failure, meconium plugs, congenital hypothyroidism, and spontaneous corneal perforation with Peter's anomaly; the infant expired on day 13. Autopsy revealed truncus, right-sided aortic arch, bilateral absence of interlobular fissures, thyroid agenesis, large horseshoe kidney with duplicated right ureter, focal glomerulosclerosis, hemivertebrae T8-T12, small cerebellum with hypoplasia of the vermis, and facial dysmorphisms.

It is unclear whether our patient and those of Jung et al. fall into the spectrum of Peters' Plus syndrome, or whether they represent a separate disorder distinguished by the presence of thyroid hypo/agenesis.

Program Nr: 1906 from the 1999 ASHG Annual Meeting

Bilateral anophthalmia with a congenital cystic eye on the left in a child with de novo balanced translocation t(10;22)(q24;q13). *M.M. Penttinen[1], K. Lahdes[2], V. Juvonen[3]*. 1) Clinical Genetics Unit, Turku Univ Central Hosp, Turku, Finland; 2) Dept. of Ophthalmology, Turku Univ Central Hosp; 3) Dept. of Medical Genetics, Univ of Turku.

Congenital cystic eye is a rare isolated or syndromic eye malformation with heterogeneous etiology. We describe a 3-month-old infant with bilateral anophthalmia, left sided cystic eye, and de novo balanced translocation t(10;22)(q24;q13). She was born to unrelated, young and healthy parents with unremarkable family history after an uneventful pregnancy at 39 week's gestation. The birthweight was 3660 g and the length 50 cm. The Apgar scores were 9/9/10. At birth bilateral eye malformations were noticed. She did not have either palpable globes or ocular movements. Her left orbit was filled with a large bluish transilluminating mass distending the upper eyelid and on the right she had only a small area of pigmented tissue at the bottom of the socket. She also had supernumerary nipples but no other physical abnormalities. Except of absent vision her growth and development have been within normal limits. MRI of brain as well as abdominal ultrasound examination were normal. Cytogenetic studies with GTG banding showed a balanced translocation t(10;22)(q24;q13) in her peripheral blood. The parents were cytogenetically normal. A search for putative candidate genes around the chromosomal breakpoints revealed that the paired box homeotic gene 2 (PAX2) previously associated with renal-coloboma syndrome has been localized at 10q24.3-q25.1. Molecular studies on the patients and her parents DNA samples however revealed no major rearrangements using the full-length PAX2 cDNA probe.

Program Nr: 1907 from the 1999 ASHG Annual Meeting

Clinical features of Coffin-Siris syndrome. *B.J. Peters[1], A. Pandya[1], L. Vanner[1], K. Kerkering[2], J.N. Bodurtha[1].* 1) Department of Human Genetics, Medical College of Virginia, Richmond, VA; 2) Department of Pediatrics, Medical College of Virginia, Richmond, VA.

We have characterized the phenotypic variability of Coffin-Siris syndrome in 18 new cases and 62 additional cases from the literature. We catalogued the features noted in previously reported cases and conducted a questionnaire study of patients ascertained through an international support group. The most frequent findings in both groups include some degree of mental retardation or developmental delay (99%), coarse facial features (88%), feeding difficulties (90%), frequent infections (87%), hirsutism (91%), abnormal or delayed dentition (96%), hypoplastic to absent fifth fingernails (95%), and hypoplastic to absent fifth distal phalanges (97%). Several other features have been noted in the neurologic, ectodermal, skeletal, gastrointestinal, and cardiovascular systems including hypotonia, sparse scalp hair, short stature, spinal anomalies, intestinal anomalies, and cardiac defects. Developmental delays are global, with the most significant delay in expressive language skills.

The etiology of this condition is unknown, though autosomal recessive inheritance has been inferred from reports of affected siblings and/or parental consanguinity in some cases. There have also been two reports of children with chromosome translocations involving chromosomes 12 and 14 and 1 and 7 respectively. Possible explanations for Coffin-Siris syndrome include etiologic heterogeneity with recessive and sporadic cases, oligogenic inheritance with a correspondingly low recurrence risk, multifactorial transmission or possibly a microdeletion involving a specific region of the genome.

Program Nr: 1908 from the 1999 ASHG Annual Meeting

Costello syndrome: report of eight patients including one with a rhabdomyosarcoma. *N.M. Philip[1], S. Sigaudy[1], D. Lacombe[2], G. Vittu[3], A. David[4], J. Vigneron[5], A. Moncla[1].* 1) Dept de Genetique Medicale, Hosp d'Enfants de la Timone, Marseille, Cedex 5, France; 2) Service de Genetique, Groupe Hospitalier Pellegrin, Bordeaux cedex, France; 3) Service de Neonatologie, centre Hosp Feron-Vrau, Lille cedex, France; 4) Service de Genetique Medicale, Hotel-Dieu, Nantes cedex 1, France; 5) Maternite Regionale A Pinard, Nancy cedex, France.

Costello syndrome is now a well delineated congenital disorder, characterized by post-natal growth retardation, dysmorphism and mental retardation. Most cases are sporadic and a significant increase of mean paternal age has been reported, suggesting de novo dominant mutations. Besides papillomata, which were part of the initial description, patients tend to develop benign tumors of ectodermal origin. We report eight new patients with typical clinical findings and emphasize the importance of cardiac manifestations and the tendency to develop tumors. Seven out of the eigth children exhibited hypertrophic cardiomyopathy. One patient developed an embryonal rhabdomyosarcoma. Occurence of that type of tumor has already been reported twice in the literature. We suggest there might be a causal link between the development of such a rare tumor and this genetic disorder. A point mutation or a microdeletion in a tumor-suppressor gene could be involved in Costello syndrome.

Program Nr: 1909 from the 1999 ASHG Annual Meeting

Poland-Moebius syndrome and symptomatic cardiac arrhythmia. *M.E. Pierpont[1], P. Hesslein[1], D. Martin[1,2], N.J. Mendelsohn[1,2].* 1) Dept Pediatrics, Univ Minnesota, Minneapolis, MN; 2) Medical Genetics Program, Hennepin County Medical Center, Minneapolis, MN.

Poland-Moebius syndrome is a well-documented association of both Poland and Moebius syndromes. It is felt to represent a formal genesis malformation syndrome of unknown etiology. Typically, Moebius syndrome is characterized by congenital facial nerve palsies in association with other cranial nerve palsies. Poland syndrome consists of unilateral symbrachydactyly and ipsilateral aplasia of the sternal head of the pectoralis major muscle. While the etiology of each of these syndromes is unknown, several families with multiple affected individuals have been described in recent years. Cardiovascular arrhythmias have not previously been reported with these conditions.

We describe a 3 year 6 month girl who was diagnosed with Moebius syndrome at birth. She initially presented with bilateral facial palsy, bilateral esotropia, chest asymmetry and poor feeding in the newborn period. There were no other limb malformations noted. The child did well until 3 1/2 years when her mother observed a two-month period with intermittent fatigue and poor appetite as well as several episodes of heart palpitations. During a febrile illness a rapid heart rate was confirmed. Evaluation revealed left axis deviation and a right bundle branch morphology which was compatible with a macro bundle re-entry ventricular tachycardia. The tachycardia spontaneously resolved.

The association of Poland-Moebius syndrome and ventricular tachycardia poses potential clues to the pathology of this rare condition. Several etiologies have been postulated for this syndrome, which include prenatal vascular disruption, primary hypoplasia or absence of the central brainstem nuclei, secondary brainstem nuclear degeneration, or peripheral nerve involvement. A vascular disruption pattern is most consistent with the creation of a focus for tachycardia within the ventricular tissue, as well as the other malformmations of ths child.

Program Nr: 1910 from the 1999 ASHG Annual Meeting

A novel SOX9 mutation in a case of Campomelic Dysplasia. *H.M. Prior[1], J. Giordano[1,2], S. Bamforth[2], M.A. Walter[1,2]*. 1) Dept. Ophthalmology, Univ. Alberta, Edmonton, AB, Canada; 2) Dept. Medical Genetics, Univ. Alberta, Edmonton, AB, Canada.

Campomelic dysplasia (CD) is a sporadic autosomal dominant syndrome that results in skeletal malformation and developmental abnormalities. Death usually occurs within the neonatal period as a result of respiratory insufficiencies, but life expectancy varies depending on the severity of the phenotype. XY sex reversal is frequently associated with CD, and a range of genital defects is observed in both males and females. CD has been linked to mutations in SOX 9, a member of the SOX (SRY-related HMG box) gene family. SOX 9 is postulated to be a transcription factor involved in chondrogenesis and sex determination based on its expression pattern and structure. We present the case of a CD patient with a normal 46,XX karyotype and female phenotype. We have carried out single-stranded conformation polymorphism (SSCP) analysis of DNA from this CD patient to determine if there is a mutation in SOX 9. An SSCP shift was observed for the proband in the C-terminal region of SOX9. DNA sequencing revealed a heterozygous frameshift mutation resulting from the insertion of a single guanine in nucleotide region 1450-1455. This insertion creates a mutant SOX9 open reading frame that is 201 nucleotides longer than the normal gene. It has been shown that the C-terminal region of the SOX9 protein is responsible for the transactivating ability of the protein. The frameshift identified here affects approximately half of the region required for full transactivating function. Functional analysis of this mutant protein may suggest why this particular CD patient has survived beyond infancy.

Program Nr: 1911 from the 1999 ASHG Annual Meeting

Mosaic isotetrasomy 20p in a liveborn. *T. Prosen[1], E. McPherson[1], U. Surti[1], G.R. Diggans[2], C.L. Jackson[3], S.M. Boehmer[3], M.E. Caine[3], J.H. Cummins[1].* 1) Department of Genetics, Magee-Womens Hospital, Pittsburgh, PA; 2) Division of Medical Genetics, The Western Pennsylvania Hospital, Pittsburgh, PA; 3) Reproductive Genetics, Western Pennsylvania Obstetrical and Gynecological Multispecialists, Pittsburgh, PA.

We report the first case of mosaic isotetrasomy 20p resulting in a liveborn infant. A 29 year old G 3 P 2, was referred for genetic counseling for an abnormal multiple marker screen (MMS) (risk for trisomy 18 at 1:35). A detailed ultrasound at 19 0/7 menstrual weeks that showed a 3 3/7 week discrepancy with a composite gestational age of 15 4/7 weeks, making her MMS too early to interpret. This ultrasound showed a deviated cardiac axis with a normal four-chamber view, and a dilated loop of bowel in the pelvis. A repeat MMS drawn at an appropriate gestational age was normal. Follow-up ultrasounds were notable for Dandy-Walker malformation, bowed long bones, and thickened nuchal fold. Fetal echocardiogram revealed dextrocardia with a significant conotruncal abnormality. Amniocentesis was initially declined but was performed at 24 weeks gestation. Results returned as 54% (23 cells) 46,XY and 46% (27 cells) 47,XY,+i(20)(p10). Parental bloods were normal. A normally grown fetus (2320 g, 45 cm length) was delivered by c-section at 35 weeks gestation (by 15 4/7 week ultrasound not consistent with LMP-39 2/7) for breech presentation in labor. Newborn exam was notable for multiple anomalies including dysmorphic features, cleft palate, excess nuchal skin, bilateral equinovarus deformities, rectus diastasis, as well as an abnormal neurologic examination. Echocardiogram revealed a double outlet right ventricle with VSD and abnormal pulmonary venous return. Complications in the newborn period included difficulty with ventilation and nonoliguric renal failure. Support was withdrawn on day two. Autopsy was declined. Chromosome studies included cord blood, 26% (13 cells) 46,XY, 74% (37 cells) 47,XY,+i(20)(p10); placenta, two sections, (100 cells) 46,XY; umbilical cord, one section, (52 cells) 47,XY,+i(20)(p10); and blood from the infant, 30% (15 cells) 46,XY, 68% (34 cells) 47,XY,+i(20)(p10) and 2% (1 cell) 48,XY,+i(20)(p10),+mar.

Program Nr: 1912 from the 1999 ASHG Annual Meeting

A new family with Trismus-Pseudocamptodactyly prompts the search for a gene in this unique form of distal arthrogryposis. *V.K. Proud, S.E. Joyner, W.S. Surka.* Div. of Medical Genetics, Dept. of Pediatrics, Children's Hosp. of The King's Daughters and Eastern Virginia Medical School, Norfolk, VA.

Trismus-pseudocamptodactyly (TPS, Dutch-Kentucky or Hecht syndrome) is an autosomal dominant condition characterized by finger curvature at the interphalangeal joints on wrist dorsiflexion due to short forearm flexor muscle-tendon units and short leg muscles producing foot deformitites. Facial anomalies include short jaw muscles, coronoid hyperplasia, reduced interincisor distance, and occasionally fibrous-like bands between the maxilla and mandible. Literature review revealed at least eleven families and approximately 314 affected individuals reported since the article by Hecht and Beals (1969). No putative genes have yet been identified and analysis using blood group markers in one family failed to show linkage to any chromosome (Robertson et el., 1982). To our knowledge, no further molecular mapping studies have been reported. Case Report: A three-generation family with seven individuals affected with TPS was evaluated when a seven year old male was referred for hand posturing, contractures, chorea, and dystonia. The unusual hand posturing was first noted at eight months of age. Development was otherwise normal. On physical exam, he had jaw muscle tightness, decreased interincisor distance, and flexion contractures of interphalangeal joints during wrist dorsiflexion. However, full extension of the digits during wrist volarflexion confirmed pseudocamptodactyly. Other family members demonstrated variable expressivity of these features, including an uncle with trismus-related anaesthetic complications. Review of Online Mendelian Inheritance in Man, OMIM™ and The Genome Database, GDB™ identified a number of distal arthrogryposis conditions. One disorder, Freeman-Sheldon syndrome (FSS), has key features that are similar to TPS, including microstomia with a puckered mouth and camptodactyly with ulnar deviation. The locus for FSS is at 11p15.5. This family will be valuable in order to complete the molecular studies that will determine if these two disorders are allelic.

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Program Nr: 1913 from the 1999 ASHG Annual Meeting

Clinical genetic study and treatment of Traditional Chinese Medicines for HEREDITARY SPASTIC PARAPLEGIA. *y. qi, h.q. lu, y.d. chen.* clinical genetics, qingdao second people's hospit, qingdao, shandong, china.

Our clinical studies are based on families referred to our genetic clinic for counselling. Regulating a balance of Yin and Yang within the human body is our principle of treatment. Hereditary Spastic Paraplegia (HSP) is a degenerative disorder of the motor system, defined by progressive weakness and spasticity of the lower limbs. HSP is inherited as an autosomal dominant (AD), autosomal recessive (AR), or an X-linked trait. We have investigated four families including 18 affected individuals. The symptomatic patients were clinically diagnosed mainly by physical examination. For diagnosis, we have used Magnetic Resonance Imaging (MRI), Computerized Tomography (CT), X-ray, Ultrasound scan, Electrocardiogram (ECG), Electromyogram (EMG), and etc. We have also performed some biochemical detection and cytogenetic analysis. Pedigree and phenotypic analysis were performed. The histories of three families suggest that their inheritance is an autosomal dominant, namely, AD-HSP, and one family is autosomal recessive. The clinical features of probands have shown late-onset HSP. The chromosome of the patients are all normal and the affected individuals' intelligence are normal. We adopted traditional Chinese medicine(TCM)in addition to routine western medicines for treatment of patients in two families. We have used Chinese medicinal herbs such as corktree, saline cistanche, Chinese atractylodes, to treat the patients. We also gave the patients acupuncture and moxibustion therapy. The major acupuncture points we applied include hegu, sanyinjiao, taichong, and baihui. Treatment is effective, the clinical symptoms of some patients have improved, their mental state and the spasticity of the lower limbs are all better than before treatment. TCM has the advantage over routine western medicines and has fewer side effects. We'll coutinue to use this approach for future treatments in a hope to improve patients' quality of life. Our plan for next study is to identify the molecular changes during the treatment of HSP with TCM.

Program Nr: 1914 from the 1999 ASHG Annual Meeting

Anophthalmos-microphthalmos spectrum: characterization of ophthalmic, systemic and genetic features in a large series. *N.K. Ragge, A.J. Vivian, R.B. Jones, J.R. Collin.* Moorfields Eye Hospital, London, England.

Anophthalmos (absence of eye) is an extremely rare congenital anomaly occurring in 0.3-0.6/10,000 births. We reviewed 83 cases of anophthalmos (or extreme microphthalmic remnant), characterising the ocular, systemic and genetic features.

There was a slight predominance of females in the series (44:39). Anophthalmos was unilateral in 28 and bilateral in 55 cases, often asymmetric. A larger proportion of males (29/39) than females (18/44) had bilateral anophthalmos. Systemic abnormalities were present in around 65% of unilateral and bilateral cases, and about the same proportion of males and females. Four patients had lid colobomas: one with a lid coloboma with microphthalmos on one side and cryptophthalmos on the other side, suggesting that these lid anomalies may form part of a spectrum.

Systemic findings included: cleft lip and palate, preauricular skin tags, facial clefting, dysplastic kidneys, corpus callosal dysgenesis, Delleman's syndrome, CHARGE, skin loss over chest wall, and branchio-oculo-facial syndrome. Possible etiologies included gestational exposure to varicella, carbamazepine and i.v. drug abuse. Autosomal recessive inheritance was implied in five cases which were the product of consanguinous marriages. One case had a strong family history of Waardenburg's syndrome. Seven cases had a family history of developmental eye anomalies or early onset blindness and 13 cases had a family history of relevant systemic features, such as deafness or cleft palate. The case with brachio-oculo-facial syndrome had an affected sibling and a cousin with 'cat-eye' phenotype. Only one case was known to have a chromosome anomaly - a Turner's mosaic. Seven families had a history of miscarriage.

This series adds significantly to the world literature on anophthalmos and further clinical and genetic analysis may provide clues to the developmental ocular genetic process.

Program Nr: 1915 from the 1999 ASHG Annual Meeting

A New Holoprosencephaly Syndrome with Major Cerebro-Oro-Facial Malformations. *A. Rajab[1], S.M. Al-Khusaibi[1], A. Riaz[1], D. Da Costa[1], A. Nair[1], M.G. Pai[1], G. Paul[1], H.A. Ghazal[1], M. Khaburi[1], A. Wasti[1], A.I. Darwish[1], T.I. Farag[2]*. 1) Clinical Genetics, Muscat, Sultanate of Oman (email: drarajab@gto.net.om); 2) Dalhousie University, Halifax, NS, Canada (email: tfarag@is.dal.ca).

Holoprosencephaly (HPE), the commonest developmental defect in the forebrain and midface, is anatomically, clinically, and etiologically a heterogeneous disorder. It has been reported in more than 70 syndromes in association with different tratogenic agents and genetic factors (chromosomal and monogenic).

We report a five-month-old girl, born at term to non-consanguineous Arab parents, with multiple major cranio-facial anomalies suggestive of a new holoprosencephalic syndrome. She was noted to have microcephaly, plagiocephaly, trigonocephaly, marked facial asymmetry, triopthalmia, hypotolerism, proboscis, macrostomia, midline V-shaped upper gum clefting, duplicated tongue with interconnecting synychia, and 46 XX, karyotype. MRI examination showed holoprosencephaly, porencephaly, lissencephaly and corpus callosum agenesis. The baby has developmental delay with intermittent febrile convulsions. Molecular analysis is currently in progress for better understanding this new Cerebro-Oro-Facial Syndrome.

Detailed clinical findings and literature review will be presented.

Program Nr: 1916 from the 1999 ASHG Annual Meeting

Congenital melanocytic hairy nevi in the genetic clinic. *J.D. Ranells[1], B.G. Kousseff[2]*. 1) Pediatrics/Genetics, University of South Florida; 2) same.

Congenital melanocytic hairy nevi (CMHN) have an estimated prevalence of 1:20,000. Thus, as rare dermal hamartomas they require adherence to strict diagnostic criteria, (J Am Acad Dermatol 24:747). Without that and reliable molecular diagnostic markers, confusion ensues; the several names used interchangeably underline that: giant hairy nevus, garment hairy pigmented nevus, nevus pigmentosus et pillosus, nevomelanocytic nevus, melanotic nevus, CMHN, Becker hairy nevus, neurocutaneous melanosis and heredofamilial melanosis (Van Bogaert syndrome). The majority of CMHN are sporadic yet familial cases have implied autosomal dominant and recessive modes of inheritance. The latter is disputed by reports of monozygous twins discordant for CMHN. To reflect on these issues we reviewed retrospectively the 18 patients with CMHN at the USF Genetic Clinic. The patients were seen between 1-2-82 and 12-31-98; they were part of the evaluated 35,095 were part of propositi/families. By location and size 12/18 were giant CMHN, 11/12 had satellite lesions and partial excision. One of them had a father with a Becker hairy nevus. Another had NF1 and CMHN with Becker hairy nevus within. One succumbed to cervical cord malignant astrocytoma at age 18 months. Among the 6/18 with moderate size CMHN, 1 had satellite lesions and another had hyperpigmented moles; the latter developed malignant melanoma within the CMHN and did well with excision. 2/6 had solitary CMHN in addition to osteogenesis imperfecta type I and neurofibromatosis type 2 respectively. The latter's father had NF2 and scalp Jadassohn sebaceous nevus, an "epidermal" nevus. The remaining 2 had solitary CMHN. In this study, based on phenotype and histologic findings, CMHN showed a spectrum of hamartomas with frequent involvement of the subcutis. The 2 patients with NF indicated that CMHN are more common in NF patients. As to pathogenesis, through the paracrine growth mechanism, aberrant molecular signaling pathway (s) should be involved in the dermal dysplasia leading to the hamartomas. Peripherin,an intermediate filament, is a candidate molecule (J Cutan Pathol 24:145); this mechanism (s) is still to be deciphered. The same applies to the differentiation between compound epidermal nevi and CMHN.

Program Nr: 1917 from the 1999 ASHG Annual Meeting

Molecular definition of del (2) (q14.1q21) in a patient with a Marfanoid body habitus, early features reminiscent of Sotos syndrome and an attention deficit disorder. *D. Ravine[1], K.L. Baker[1], M.I. Rees[1,2], P.W. Thompson[1], R.T. Howell[3], T.R. Cole[4], H.E. Hughes[1], M. Upadhyaya[1]*. 1) Inst Medical Genetics, Univ Hosp Wales, Cardiff, Wales; 2) Department of Psychological Medicine and Medical Genetics, University of Wales College of Medicine, Cardiff, UK; 3) Regional Cytogenetics Centre, Southmead Hospital, Bristol, UK; 4) West Midlands Regional Clinical Genetics Service,Birmingham, UK.

We report findings in a young male with an attention deficit disorder and learning difficulties combined with a large birth weight, Marfanoid body habitus and early physical features suggestive of Sotos syndrome. Cytogenetic analysis showed an interstitial deletion of chromosome 2 (46,XY,del(2)(q14.1q21). Microsatellite and FISH analysis revealed that the deletion spanned approximately 13cM (D2S2271 to D2S2282). As the chromosome abnormality in our patient may play a role in the occurrence of the physical features found in Sotos syndrome, samples from 18 other individuals with unequivocal Sotos syndrome were assessed for evidence of microdeletions and uniparental disomy involving 2q14.1 to 2q21. Negative results were obtained. This case reveals that haploinsufficiency of this chromosomal region is responsible for intellectual impairment, a Marfanoid body habitus together with some physical features reminiscent of Sotos syndrome.

Program Nr: 1918 from the 1999 ASHG Annual Meeting

Stress urinary incontinence and menarche in women with osteogenesis imperfecta. *S.D. Reed[1,2], M.G. Pepin[3], K. Peters[3], G.M. Lentz[1], E.D. Lease[1], P.H. Byers[3].* 1) Department OB/GYN, University of Washington, Seattle, WA; 2) Gynecology Division, Harborview Medical Center, Seattle, WA; 3) Department of Medical Genetics, University of Washington, Seattle, WA.

Connective tissue alterations are thought to be important in the genesis of female stress urinary incontinence (SUI) and pelvic relaxation. Osteogenesis imperfecta (OI) is one of several heritable disorders of connective tissue with known molecular pathogenesis. To determine if mutations in defined genes might contribute to SUI and pelvic relaxation, we surveyed ~50 adult women > age 25 with OI who are members of the OI Foundation. The survey requested demographic, genetic, obstetric and gynecologic information. 35 responded (70% response rate); 2 were excluded because the diagnosis of OI was not clear or the survey was not completed adequately. Of the 33 subjects, 30 were Caucasian and 3 were Asian. Twelve had OI type I (33%), 9 - OI type III (27%), and 12 - OI type IV (33%). Four (12%) reported symptomatic SUI with loss of urine >1x/wk (OI type I- 1; OI type III-1; OI type IV- 2). Five (15%) reported pelvic relaxation (OI type I- 3; OI type III- 1; OI type IV- 1). Five (15%) had symptomatic SUI and/or pelvic relaxation and 3 of the 5 (9%) had onset at age <30. Pelvic relaxation was related to a prior term delivery, SUI was not. Early onset of SUI/pelvic relaxation was more common in women with OI and may be related to the underlying molecular defect in type I collagen genes.

We analyzed age of menarche to determine if there was a correlation of SUI and pelvic relaxation with overall decreased estrogen exposure. Age of menarche and SUI/pelvic relaxation did not correlate; however, delayed menarche (onset of menses >1 SD after the mean of 12.9 years for US girls) was seen in 42% and was increased in OI type III (55%). Age of menarche ranged from 14-23 years. Delayed menarche has been reported in women of short stature and may be due to decreased body fat or body mass index (BMI), but this was not a consistent finding in our study. Further analysis of the age of menarche in OI is warranted, especially as this affects osteoporosis risks.

Program Nr: 1919 from the 1999 ASHG Annual Meeting

Isolated palatal anomaly is not an indication to screen for 22q11 deletion. *O. Reish[1,2], R. Masterman[2], Y. Finkelstein[3], A. Nachmani[3], B. Wolach[2], M. Fejgin[1], A. Amiel[1]*. 1) Genetic Institute; 2) Department of Pediatrics; 3) Department of Otolaryngology- Head and neck Surgery, Meir Hospital, Kefar Saba, Sackler School of Medicine, Tel Aviv University, Israel.

Velocardiofacial syndrome (VCFS) is the most common syndrome associated with clefting of the secondary palate and is associated with hemizygous deletion of 22q11 region. However, patients with VCFS may present a special diagnostic challenge because the phenotype of patients with VCFS is extremely variable and these patients may present only part of the classically described anomalies. Since the frequency of the deletions in patients presenting with isolated palatal anomalies has not been fully evaluated yet, it has been hypothesised that every patient with palatal anomaly has to be screened for 22q11 deletion. This hypothesis has been verified in our study. Forty-two consecutive patients, referred to our palatal clinic of the ENT department because of velopharyngeal insufficiency and found as having isolated palatal anomalies and negative familial history were included in the study. There were 20 males and 15 women, and their age varied from 2 weeks to 31 years (mean = 12.27.4). 10 patients had overt cleft palate, 10 patients had occult submucous cleft palate and 10 patients nasopharyngeal disproportion. A cytogenetic analysis utilising FISH kit for 22q11 region (Oncor) was performed in all the patients. The analyses were all negative and thus deletion of 22q11 region was excluded in all our patients. Our conclusion is that there is no indication to screen patients presenting with isolated velopharyngeal insufficiency for deletion of 22q11 region. However, patients with palatal anomalies should be followed and re-evaluated. When additional other signs or symptoms relevant to VCFS are found including behavioural or psychiatric disorders, which may evolve at an older age deletion of 22q11 region, should be analysed.

Program Nr: 1920 from the 1999 ASHG Annual Meeting

Fryns Syndrome: association with unbalanced translocations trisomic for 20q13.1-qter in two unrelated families.

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Fryns syndrome is an autosomal recessive disorder for which the genetic defect has not been identified. Cardinal features include diaphragmatic hernia, facial dysmorphism, and digital hypoplasia, often associated with cardiac, skeletal, CNS, and GI tract malformations. We report on 2 families in whom paternally-derived unbalanced translocations involving 20q13.1-qter were associated with features of Fryns syndrome. The first infant, with prenatally-detected hypoplastic left heart, proved to have natal teeth, facial dysmorphism, hypoplastic digits, camptodactyly, and 13 ribs. Karyotype was 46,XX,der(15)t(15;20)(q26.3;q13.1). Cytogenetic studies were not performed on a male sibling who had died with diaphragmatic hernia, and rib and vertebral anomalies. The phenotypically normal father's karyotype is 46,XY,t(15;20)(q26.3;q13.1). In the second family a balanced translocation, 46,XY,t(4;20)(q34.2;q13.1), was found in the phenotypically normal father of 2 anomalous fetuses, each with the unbalanced karyotype 46,XY,der(4)t(4;20)(q34.2;q13.1). The first pregnancy was terminated at 18 weeks. Diaphragmatic hernia, omphalocele, and enlarged fourth ventricle were confirmed on autopsy. They continued the next pregnancy, despite ultrasound findings of hypoplastic left heart and enlarged fourth ventricle. Fetal demise occurred at 27 weeks. Autopsy was declined. External inspection of the macerated fetus found facial dysmorphism (broad flat nose with bifid tip;flat simple pinnae). Unbalanced translocations with breakpoints at 20q13.1 produced similar constellations of malformations in two unrelated families. While these cases may represent phenocopies of Fryns syndrome, it is also possible that a gene localized to the breakpoint is responsible for the syndrome. Molecular analysis of the breakpoint region may help delineate the genetic defect in Fryns.

Program Nr: 1921 from the 1999 ASHG Annual Meeting

A new neurological syndrome with mental retardation, choreoathetosis, and abnormal behavior maps to chromosome Xp11. *E. Reyniers[1], N. Peeters[1], P. Van Bogaert[2], N. Van Regenmorter[2], R.F. Kooy[1]*. 1) Dept Medical Genetics, University of Antwerp, Antwerp, Belgium; 2) 1Erasmus Hospital, Free Brussels University, Brussels, Belgium.

We describe a new X-linked neurological syndrome, characterized by mild mental retardation, choreoathetosis, and abnormal behavior. Choreoathetosis, the most distinguishing feature of those patients, is a dyskinesia characterized by attacks of involuntary rhythmic and arrhythmic movements referred to as chorea and athetosis, respectively. It occurs in a variety of clinically and genetically diverse disorders, albeit rarely in familial forms. Clinically, the disease bears some resemblance to Lesch-Nyhan syndrome (mapped to Xq28). However, we mapped the disease in our family to chromosome Xp11 by genetic linkage analysis, we defined a novel X-linked syndrome. The region contains several interesting candidate genes that play a role in neuronal signaling, including GPR34, a neuronal G-protein-coupled receptor. G-protein-coupled receptor target molecules include small GTPases, three of which have been shown to be mutated in familial cases of non-syndromic mental retardation. Moreover, this receptor class has been suggested to play a role in behavioral and psychiatric disturbances. Mutation analysis of this gene is underway and may in the future elucidate the molecular defect in this family.

Program Nr: 1922 from the 1999 ASHG Annual Meeting

Abnormal vascular proliferation in Poland-Moebius anomaly. *M. Rittler, R. Rosetto, L. Campora, E. Altamirano, R. Fuksman.* Hospital Materno Infantil Ramon Sarda, Buenos Aires, Argentina.

Poland anomaly consists of congenital absence of the pectoralis major muscle with a variable degree of upper limb hypoplasia. Moebius anomaly comprises facial nerve palsy with or without other cranial nerve involvement. When present, the most frequently reported CNS anomalies were hypoplasia or focal lesions of the brainstem or the cranial nerve nuclei. Both conditions frequently overlap and are considered, among others, as part of the subclavian artery supply disruption sequence. Classically, it is considered of vascular pathogenesis, though the underlying cause is unclear and possibly heterogeneous. Many reports suggest an association between Poland-Moebius anomalies and exogenous teratogenic maternal exposures, but familial recurrence has also been reported and syndromes of recognized genetic etiology as Adams-Oliver, Marden-Walker and Carey-Fineman-Ziter also overlap with this spectrum. We report on a newborn with clinical features of Poland and Moebius anomalies. The autopsy revealed replacement of the left pectoralis major muscle and bilateral diaphragms by a thin, fibrous membrane, and pulmonary hypoplasia. CNS findings included enlargement of the lateral ventricles and small third and fourth, with partial aqueductal stenosis; convolutional underdevelopment, absence of the corpus callosum, hypoplasia of the cerebellum, and macroscopically visible focal anomalies of the brainstem. Microscopically, there was proliferation of abnormally shaped vessels, predominantly in leptomeninges, brain, cerebellum and brainstem, and periependymal foci of hemorrhage and calcification. Though the mentioned anomalies could all be due to an unnoticed teratogen, the vascular abnormality in our patient could also be considered as a primary structural defect, leading to secondary hemorrhage, calcification and brain involution.

Program Nr: 1923 from the 1999 ASHG Annual Meeting

Cognitive functioning in Prader-Willi syndrome: Comparison of genetic subtypes. *E. Roof[1], W. Stone[1], W. MacLean[2], I. Feurer[1], T. Thompson[1], M.G. Butler[3].* 1) John F. Kennedy Center, Vanderbilt University, Nashville, TN; 2) University of Wyoming, Laramie; 3) The Children's Mercy Hospitals and Clinics, Kansas City, MO.

Prader-Willi syndrome (PWS) is a classical genetic condition with two distinct genetic subtypes [paternal deletion of 15q11-q13 region in about 70% of subjects and maternal uniparental disomy (UPD) of chromosome 15 in about 30% of subjects] and unusual behavioral characteristics. PWS is characterized by infantile hypotonia, hypogonadism, feeding difficulties, early childhood obesity, short stature, small hands and feet, mental deficiency and a particular facial appearance. Hypopigmentation is a recognized feature in deletion PWS subjects compared with those with UPD; however, recent advances in genetics have led to an increased understanding of the role of the genotype on behavioral functioning. The purpose of the present study was to examine differences in intellectual functioning in individuals with PWS with the 15q11-q13 deletion versus those with UPD. Measures of intelligence and academic achievement were administered to 38 individuals with PWS [24 deletion (9 males, 15 females) and 14 UPD (7 males, 7 females) between 10 and 50 years of age with a mean age of 21 years]. Data demonstrated that persons with UPD had significantly higher verbal IQ scores than those with a deletion. The magnitude of the difference in verbal IQ was 9.1 points (69.9 vs 60.8 for UPD and deletion PWS subjects, respectively). Only 17% of persons with the 15q11-q13 deletion had a verbal IQ > 70 while 50% of those with UPD had a verbal IQ of > 70. This is the first report to document the difference between verbal and performance IQ score patterns among persons with PWS of the deletion versus UPD subtype.

Program Nr: 1924 from the 1999 ASHG Annual Meeting

Symetric or asymetric limb deficiencies in multiply malformed children. *A. Rosano[1], C. Stoll[2]*. 1) ICBDMS, Via Sabotino, Roma, Italia; 2) Service de Genetique Mdicale, Hopital de Hautepierre, Strasbourg, France.

In human beings, the unpaired organs are placed in a highly ordered pattern along the left-right axis.Rarely people are born with complete or incomplete mirror-image reversal of this intricate asymmetry of the visceral organs.How are the organs normally patterned with such precise left-right asymmetry, and what goes awry when they are mispatterned? A cascade of signalling molecules regulating the establishment of embryonic left-right asymmetry is indicated by recent studies of chick and mouse embryonic development.This cascade includes Sonic hedgehog (Shh), nodal (a memeber of the TGF family), the snail-related (cSnR-1) zinc-finger transcription factor, lefty, activin-receptor, cubitus interruptus (Ci), decapentaplegic (dpp) and ZIC proteins, encoding a zinc-finger transcription factor.It has been identified that some of these genes are involved in limb patterning.Therefore a way trying to answer to the abovementionned question is to verify in multiply malformed children, if there is symmetry or asymmetry regarding to the limb defects. The material for this study are the cases with multiple congenital anomalies (the multiple) with limb defect (LD) collected by the International Clearinghouse for Birth Defects Monitoring Systems (ICBDMS);analysis of laterality was performed on 666 non syndromic cases of LD associated with other major malformations. In this material anomalies of the left arm and leg were compared to anomalies of the right arm and leg regarding to the extra limb anomalies present in these multiple i.e congenital heart disease, gut, liver and spleen anomalies.Severe genitalia defects were more often associated to left LD than to right LD (OR=2.6, CI 1.1-6.4).Gastroschisis and axial skeletal defects were more often associated to unilateral than to bilateral LD (OR=9.2, CI 1.2-70.3 and 1.9, CI 1.1-3.0, respectively).Comparisons left versus right by type of LD were not significant in this material.However cleft lip/palate was more often associated with bilateral preaxial LD, gastroschisis with bilateral transverse LD and with amelia and urinary tract defect with amelia.The distribution of LD laterality was also studied in syndromic cases.

Program Nr: 1925 from the 1999 ASHG Annual Meeting

Familial Beare-Stevenson syndrome attributable to a P250R missense mutation in the Fibroblast Growth Factor 3 Receptor (FGFR3). *T. Roscioli[1], S. Flanagan[2], P. Kumar[2], J. Masel[3], R. Lewandowski[4], V. Hyland[2], I.A. Glass[1]*. 1) Queensland Clinical Genetics, Royal Children's Hospital, Brisbane, QLD Australia; 2) Royal Brisbane Hospital Molecular Genetics Laboratory; 3) Radiology Department, Royal Children's Hospital, Brisbane; 4) Craniofacial Unit, Mater Children's Hospital, Brisbane.

Beare-Stevenson Syndrome (BSS), is an autosomal dominant condition comprising craniosynostosis, cutis gyrata, acanthosis nigricans and other anomalies. At least five patients, to date all sporadic, have been described since the initial report in 1969. A proportion of these BSS cases have been found to have mutations in the linker region between the third immunoglobulin-like domain and the transmembrane domain of FGFR2 or the transmembrane domain of FGFR2. Our female proband was born with a severe and progressive turricephaly, a birthweight of 3.4kg. (90th percentile) and a head circumference of 31cm. (3rd percentile). Reflection of the abdominal skin onto the umbilical cord, typical of BSS, was present. Cutis gyrata developed on the palm of the left hand, which became more prominent over the following months. Deep furrowing on both soles and a linear patch of acanthosis nigricans on the left forearm were also noted. The probands father had broad thumbs and brachydactyly and there was cutis gyrata present on his forehead as well as radiological evidence of hypertelorism. Molecular analysis of the FGFR3 gene was carried out by PCR and Nci1 digest. This demonstrated an FGFR3, P250R missense mutation was present in one allele in both the proband and her father. This is yet another example of an activating mutation in a FGFR gene which causes a common phenotype between FGFR syndromes, in this case BSS. The lack of familial examples of BSS with FGFR2 mutations suggests this mutation has a significant impact on reproductive fitness.

Program Nr: 1926 from the 1999 ASHG Annual Meeting

Clinical spectrum of (ponto)cerebellar hypoplasia with anterior horn cell involvement. *S. Rudnik[1], T. Voit[2], L. Sztriha[3], B. Wirth[4], K. Zerres[1]*. 1) Institute for Human Genetics, Technical University of Aachen, Germany; 2) Department of Pediatrics, University of Essen, Germany; 3) Department of Pediatrics, UAE University Al Ain, United Arabic Emirates; 4) Institute for Human Genetics, University of Bonn, Germany.

(Ponto)cerebellar hypoplasia (PCH) is rarely associated with spinal muscular atrophy (SMA) and designated as PCH-1. This phenotype is characterized by severe muscle weakness and hypotonia starting prenatally or at birth with life span not exceeding a few months in most cases. We herewith describe the clinical and pathological findings in 9 families with evidence of cerebellar defects and SMA, representing a hitherto unknown spectrum of clinical variability. In all patients, the diagnosis of SMA was made on the basis of electrophysiological data and muscle biopsy, however, genetic testing failed to confirm the diagnosis of infantile SMA I-III with a gene defect on chromosome 5q and resulted in clinical reevaluation. Four isolated patients had a picture resembling SMA II or III and developed cerebellar atrophy/hypoplasia later. Age at onset was around the first birthday in 3 patients and at 6 years in 1 patient. Last information was obtained at 3-7 years, 1 patient died at 16 years. Five families had an early onset prenatally or shortly after birth with a severe phenotype which might be mistaken for SMA type I (Werdnig-Hoffmann disease). Life span did not exceed a few months in 4 families, whereas 2 sisters died at 16 and 26 months, and 1 sibship survived 4 years. The genetic basis of cerebellar hypoplasia plus SMA remains to be determined, parental consanguinity and affected sibs make autosomal recessive inheritance most likely. Molecular genetic studies allowed to exclude the gene locus for infantile SMA in some families tested.

Program Nr: 1927 from the 1999 ASHG Annual Meeting

Neonatal Incontinentia Pigmenti in an affected male within a normal family. *S. Rugolotto[1], F. Soli[2], M. Benedetti[1], C. Ghizzi[1], P. Biban[1], A. Turco[2], P.F. Pignatti[2]*. 1) Pediatric Intensive Care Unit, Mayor Civil Hospital, Verona, Italy; 2) Section of Biology and Genetics, Mother and Child Department, University of Verona, Verona, Italy.

Incontinentia Pigmenti (IP) is a rare X-linked dominant genodermatosis. Prevalence and incidence in the population are unknown. About 97% of the patients are females. We describe an affected newborn whose family was normal. He was a term baby born to a para 0000, by cesarean section due to fetal distress. APGAR score 8(1') - 9(5') was given. Birth weight was 2670 g, length 47.5 cm, head circumference 33.2 cm. Maternal serum tests for HBsAg, CMV, HSV, HIV, HCV, VDRL, toxoplasmosis were negative and for rubella IgG positive. Family history was negative for skin, neurologic disorders and abortions. At birth erythematous blisters were scattered over the body surface, particularly on the upper and lower extremities, according to a linear distribution and on the back and the armpit in a swirly pattern. Mucosae were normal and dysmorphic traits were absent. C-Reactive Protein, blood culture, skin swab, WBC were normal. At 10 days of life a skin biopsy was consistent with IP. Thus we performed cardiac, head, and abdominal ultrasound, EEG, chest X-Ray, and eye exam which were all normal. The Karyotype was 46, XY. The subsequent neonatal growth pattern was normal. At 30 days of life several skin lesions resolved without scars and some warty, hyperkeratotic lesions were evident on the hands. At 3 months of age the infant was growing and feeding well and the warty lesions were not evident any longer. At present we have observed the first two stages of the disease. Follow-up is needed to evaluate stage 3 (macular hyperpigmentation), stage 4 (hypopigmentation with slightly atrophic skin), which both occur later and to treat possible dental, eye, and neurologic abnormalities which might affect IP natural history. One IP gene has been mapped in Xq28 but it has not been identified yet. The basic defect of IP remains unknown. Karyotypically normal male patients within normal families are very rare, and they might be helpful for a better understanding of this disorder, should we be able to identify the IP gene.

Program Nr: 1928 from the 1999 ASHG Annual Meeting

Neonatal respiratory death in the 22q11.2 deletion. *K.L. Russell[1], D.M. McDonald-McGinn[1], W. Mahle[1], L. Howell[1], S. Kasperski[1], B.M. Wilkes[2], J. Meyer-Cohen[2], S. Adzick[1], E.H. Zackai[1].* 1) The Children's Hospital of Philadelphia, Philadelphia, PA; 2) Medical University of South Carolina, Charleston, SC.

The 22q11.2 deletion has been identified in most patients with DiGeorge, VCFS, & CTAF and in some cases of Opitz G/BBB and Cayler cardiofacial syndromes. We found a 5% mortality rate in 305 patients with the 22q11.2 deletion, 90% due to heart disease. Here we report two additional patients with the deletion who succumbed to respiratory complications shortly after birth. Patient 1 presented at 25 weeks gestation with Apgars of 4 and 5. Endotracheal intubation was unsuccessful and the patient expired. Post mortem exam revealed tracheal agenesis with fused main bronchi, bronchoesophageal fistula, and heterotaxia. Minor dysmorphia was noted including small ears with overfolded helices and a bulbous nasal tip. Chromosome analysis was suggested. In addition, 22q11.2 deletion studies were performed based on the association of laryngotracheoesophageal abnormalities (McDonald-McGinn et al. 1995). Subsequent autopsy findings included complete AV canal, posterior malaligned VSD, bicuspid aortic valve, IAA type B, and absent thymus. Karyotype was normal but the patient had a 22q11.2 deletion detected by FISH. Parental studies revealed a deletion in the father as well. Patient 2 presented prenatally with a left-sided diaphragmatic hernia on ultrasound. No other anomalies were noted at that time. Amniocentesis was normal. A subsequent consultation at our fetal therapy center included an echo, which revealed TOF. Deletion studies were suggested based on the association in patients with TOF (15% Goldmuntz et al. 1999). The pregnancy continued without further studies and the patient was born at 39 weeks gestation. The child died at 24 hours from respiratory failure. Post mortem FISH analysis revealed a 22q11.2 deletion. Review of the literature did not reveal any other cases of either tracheal agenesis or CDH in patients with the 22q11.2 deletion. However, we have one additional case of a small CDH in a child who survived surgery. Thus, we suggest that deletion studies be considered when either of these anomalies are present in the face of associated manifestations of the 22q11.2 deletion.

Program Nr: 1929 from the 1999 ASHG Annual Meeting

A new syndrome with large hands, foot deformities, and scoliosis maps to Xq24-26. *D. Salazar[1], A. Vaglio[2], R. Quadrelli[2], S. Reyno[2], A. Lemes[2], R.S. Lachman[1], W.R. Wilcox[1]*. 1) Medical Genetics, Cedars-Sinai Med. Cntr., Los Angeles, CA, USA; 2) Instituto de Genetica Medica, Hosp. Italiano, Montevideo, URUGUAY.

We report a 3 generation family with an apparently previously unreported X-linked recessive skeletal dysplasia. There are 7 affected males. Among the females, there are 3 obligate carriers who have mild manifestations of the disorder or are clinically normal. Although there is no male-to-male transmission, there have been few offspring of affected males. Affected males have a large neck, large palms, scoliosis, prominent muscular development, and progressive joint dislocations and pes cavus deformities in the feet. Radiographs show carpal and tarsal fusions, joint dislocations and secondary osteolysis in the feet. Variable features in males include camptodactyly in the hands, congenital hip dislocation, and degenerative osteoarthritis of the hips. This condition has some similarities with, but is clearly distinct from, two other X-linked disorders, frontometaphyseal dysplasia and otopalatodigital syndrome type I.

Genetic analysis using 18 polymorphic markers along the X-chromosome was performed. Recombinational events were used to narrow the most likely disease gene containing region to a 25 cM interval bounded by the markers DXS424 and DXS8072, corresponding to cytogenetic bands Xq24-26.

Program Nr: 1930 from the 1999 ASHG Annual Meeting

A New Association of Kabuki Make-up syndrome and Malignant Hypertermia. *A.A. Santa Rosa[1], J.C. Llerena[1], R.T. Sudo[2], J.C. Cabral de Almeida[1]*. 1) Centro de Genetica Medica, Instituto Fernandes Figueira/FIOCRUZ, Rio De Janeiro, RJ, Brazil; 2) Centro de Hipertermia Maligna, Dept. Farmacologia, ICB/UFRJ, Rio de Janeiro, RJ, Brazil.

Kabuki Make-up syndrome (KS) is characterized by unusual facies, (oriental gestalt) mild to moderate mental retardation, skeletal abnormalities, dermatoglyphic alterations and postnatal growth retardation. We present a nine year-old boy, referred due to facial dysmorphisms. His parents were third-degree cousins. The diagnosis of KS was made at the first admission, for he had low palpebral eversion, high-arched palate, myopia, ptosis, brachymesophalangy, fetal pads and bilateral cryptorchidism. Cytogenetic analysis was normal and no other anomalies were found. However, when he was submited to orchipexy, during the anesthesia he developed a malignant hyperthermia crisis (41,9°C), immediately reverted with dantrolene sodium IV. A muscular biopsy was performed and the sample was submited to the Kalow test (in vitro caffeine/halothane contraction test for suscetible muscle), the only widely accepted test for malignant hyperthermia (MH) The result was positive. Mutations in the RYR1 gene (19q13.1) are responsible for half of the MH cases. A microdeletion syndrome could explain the findings. To our knowledge, it is the first reported association of KS and MH. Our patient can help mapping the KS if the RYR1 gene is involved in his phenotype, for these two entities may be linked.

Program Nr: 1931 from the 1999 ASHG Annual Meeting

Reevaluation of frequencies of selected features in patients with the 18p- syndrome. *R.L Schaub[1], R.J. Leach[1,2], J.D. Cody[1]*. 1) Pediatrics, University of Texas Health Science Center, San Antonio, TX; 2) Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, TX.

The 18p- syndrome results from loss of genetic material from the short arm of chromosome 18. Mental retardation, short stature, midline defects, and ptosis have been reported previously in the literature as the most common features of this syndrome. In an attempt to better understand this syndrome, we collected medical records and blood samples from 21 individuals with de novo deletions of 18p. After completion of the chart review, we noted that our study population differed significantly from prior case reports in several respects. The incidence of hypotonia and ptosis in our group were 95% and 48%, respectively, higher than the previously reported frequencies of 23% each. Microcephaly (FOC < 3 S.D. below the mean for age) was documented in only one of our participants, in contrast to 23% in the literature. Speech delay was found in 100% of our participants; however, receptive language abilities were considered age appropriate. Although mental retardation is listed as the most common feature of this syndrome in the literature, only three of our participants met DSM-IV criteria for mental retardation (IQ<70) and none were severely mentally retarded. Molecular analysis was performed on the DNA isolated from these samples. A total of 22 chromosome 18 short arm and centromere specific markers were used for estimating the extent of the deletion of 18p and determining the parental origin of the deletion. Some individuals who were determined to have a deletion of the entire short arm had IQ scores in the 70-80 range. Our data suggest that the literature currently available on the 18p- syndrome may not describe accurately this condition and that the primary abnormality associated with 18p- syndrome may be an expressive language/speech delay rather than mental retardation. Further research ultimately may enable physicians to give families a more accurate prognosis concerning the development of their child with 18p- syndrome.

Program Nr: 1932 from the 1999 ASHG Annual Meeting

A *de novo* deletion of more than 2 Mb including the *SOX9* locus in a case of campomelic dysplasia (CD) proofs that CD is a haploinsufficiency syndrome. *G. Scherer[1], K. Lindenberg[1], J. Zimmer[1], B. Schmalenberger[2], D. Pfeifer[1].*
1) Institute of Human Genetics,University of Freiburg, Freiburg, Germany; 2) Medizinische Genetik, Passau, Germany.

Campomelic dysplasia (CD; MIM 114290) is an autosomal dominant skeletal malformation syndrome with XY sex reversal that results from heterozygous missense, nonsense or frameshift mutations in the chondrogenic regulatory gene *SOX9* on 17q. Although all *SOX9* mutations described so far are most likely loss-of-function mutations, arguing for haploinsufficiency for SOX9 product as the cause for CD, definite proof for this hypothesis has been lacking. We studied a male patient with features characteristic for CD: delayed ossification, short and bowed femora, tibiae and fibulae, narrow iliac wings, hypoplastic ischiac and pubic bones, bell-shaped thorax, 11 pairs of ribs, clubbed feet, and Robin sequence. The patient died at the age of 10 months. Chromosomes were unremarkable cytogenetically. FISH analysis with YAC 946E12 that extends from about 2 Mb upstream to 200 kb downstream of *SOX9* resulted in signals on only one of the two chromosomes 17. This deletion of more than 2 Mb was confirmed by analyzing a somatic cell hybrid clone that carried the deleted chromosome 17 in the absence of the normal chromosome 17. By PCR, this hybrid was found negative for all markers mapping on YAC 946E12 as well as for markers located proximal and distal to this YAC. The most proximal and most distal deleted markers are *D17S949* and *D17S1829*, respectively, that are 6 cM apart. Haplotype analysis revealed that the deletion was of paternal origin. A mutation in the remaining *SOX9* allele of the patient was ruled out by DNA sequencing. Together with a recent report on a similar *SOX9* deletion CD patient (Am. J. Med. Genet. 84:20-24, 1999), this case provides strong evidence for CD being caused by *SOX9* haploinsufficiency.

Program Nr: 1933 from the 1999 ASHG Annual Meeting

Anophthalmia/Microphthalmia Registry: Review of 90 cases further demonstrates heterogeneity of anophthalmia/microphthalmia. *A.S. Schneider[1], T.M. Bardakjian[1], C.M. Sellers[2], J.E. Barbagallo[1]*. 1) Dept Genetics, Albert Einstein Medical Ctr, Philadelphia, PA; 2) *Beaver College, Glenside, PA.

The Anophthalmia/Microphthalmia (A/M) registry was established in 1993 to assemble a national database of individuals with A/M, to determine the local and national incidence of A/M and to undertake a descriptive analysis of the registry population. In April 1999, an international conference on A/M was held in Philadelphia in conjunction with the support group, ican (International Children's Anophthalmia Network). This provided the opportunity for clinical evaluation of affected individuals and initiation of DNA collection for a screening initiative looking at 10 eye development genes in 3 laboratories. Our previous observation that there are more males than females with bilateral anophthalmia is again demonstrated with our larger numbers. Of the 90 cases reviewed, 48 were males (53%). Of the males, 71% had bilateral anophthalmia. There were 42 females in the group and 50% had bilateral anophthalmia. In both males and females, developmental delay was more common in those with bilateral anophthalmia, although 23% were too young to assess. A significant number of males with bilateral anophthalmia had other structural anomalies (67%) although not all males with structural anomalies were delayed. 43% of females with bilateral anophthalmia had structural anomalies. The heterogeneity of A/M noted in our registry underscores the importance of identifying the genes involved in this complex and rare malformation.

Program Nr: 1934 from the 1999 ASHG Annual Meeting

A newly recognized trigonocephaly syndrome: Fronto-Ocular Syndrome. *E.N. Schneider[1], A. Bogdanow[1], J.T. Goodrich[2], R.W. Marion[1], M.M. Cohen, Jr[3].* 1) Department of Pediatrics, Montefiore Medical Center/Albert Einstein College of Medicine, Bronx, NY; 2) Department of Neurosurgery, Montefiore Medical Center/Albert Einstein College of Medicine, Bronx, NY; 3) Department of Oral and Maxillofacial Surgeries, Dalhousie University, Nova Scotia, Canada.

Trigonocephaly can have a variety of different presentations. The common clinical features include orbital hypotelorism, ethmoidal hypoplasia and a keel-shaped, symmetric frontal bone deformity. The skull typically has a triangular shape due to forehead prominence, increased parietal diameter and temporal depression. Trigonocephaly can be isolated, syndromic or associated with other congenital anomalies. We report a mother and her daughters who share a particular constellation of clinical findings including trigonocephaly and facial dysmorphism which encompasses ocular hypotelorism, ocular proptosis and ptosis, epicanthal folds, elevated nasal bridge, thin philtrum, arched palate and a narrow bifrontal region. In addition, both daughters have glabellar capillary hemangiomas, a congenital heart defect and mild developmental delays. Review of the literature failed to disclose any similar syndrome. We believe that this is an autosomal dominantly inherited condition that arose as a new mutation in the mother.

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Program Nr: 1935 from the 1999 ASHG Annual Meeting

Congenital myotonic dystrophy: Novel phenotypic features. *R.E. Schnur, R.A. Kurnik, L.A. Reed, K.A. Mockridge, S.O. Imaizumi, B.J. Evans, S.E. Courtney, N.M. Razi, P.B. Pandit, J.G. Saslow, G.E. Stahl.* Cooper Hosp/UMDNJ at Camden.

Congenital DM is associated with phenotypic effects of hypotonia. We report novel features in 4 infants with congenital DM. Patient 1, a 2300g/38 week female (~1133 CTG repeats of myotonin protein kinase), had hypotonia, laryngomalacia, vocal cord granuloma, PFO,PPS, frontal bossing, prominent occiput, tall cranial vault, deep set eyes, epicanthi, low-set ears, bulbous nose with underdeveloped tip, small mouth, tented lip, high palate, micrognathia, delayed dentition, joint laxity, & narrow pelvis. MRI: dilated ventricles, decreased WM, & small CC. Patient 2, a 2200g/34 week male (~1633 repeats), had hypotonia, respiratory insufficiency, diaphragmatic paralysis, sepsis, macrocephaly, trigonocephaly, ridged sutures, synophrys, deep set eyes, bitemporal narrowing, asymmetric shield chest, small eyes, hypotelorism, epicanthi, bulbous nose/anteverted nares/underdeveloped tip, small mouth/thin/tented lip, high palate, micrognathia, anomalous ears, 11 ribs, undescended testis, hypospadias, sacral cleft, clubfeet, clinodactyly, single palmar crease, PDA, & ASD. MRI: dilated lateral ventricles, choroid plexus hemorrhage, metopic & coronal craniosynostosis. Patients 3 & 4 were monozygotic AGA twins, born @ 29 weeks (~1067 repeats each). Both had fused eyelids that did not open until ~10 days, hypotonia, sepsis, respiratory distress, small palpebral fissures, prominent nose, anteverted nares/underdeveloped tip, small mouth, micrognathia, bell-shaped thorax, long fingers, prominent heels, high arches, & cryptorchidism. Only twin B survived; at 4 months, length/weight were <5th%ile; head circumference was relatively large at ~50th%ile. Summary: All had typical congenital DM + distinctive facies: bulbous nose, deep-set eyes/fused eyelids, small mouth, & macrocephaly. They also had other anomalies: congenital heart disease, missing rib, laryngomalacia, CNS anomalies, & craniosynostosis. Besides neonatal hypotonia & family history of DM, multiple anomalies, facial dysmorphia, & fused eyelids at a late gestational age are indications for DM testing. **Patient 1Patient 2Patients 3 and 4Summary**.

Program Nr: 1936 from the 1999 ASHG Annual Meeting

Deletion of chromosome band 7q33 results in a syndrome of mild to moderate mental retardation and mild dysmorphic features: a new contiguous gene deletion syndrome? *S.A. Schonberg, C.J. Tifft.* Children's Nat'l Medical Center, George Washington University School of Medicine Department of Pediatrics, Washington, DC.

We report two children with a 7q deletion limited to band 7q33 [del(7)(q32q34)]. This deletion has been reported only very rarely, possibly due to the subtlety of both the cytogenetic findings and the clinical features. We compare our cases to the four other cases in the literature (3 from a single family) and propose that this deletion may define a new contiguous gene deletion syndrome.

Case 1, a 4-year old female, was referred due to developmental delay and Case 2, also female, age 3, was referred neonatally after the de novo deletion had been detected at anmiocentesis performed for an abnormal maternal serum screen. She represents the first unbiased ascertainment for this deletion. She is mildly retarded. Shared physical features, inclusive of previously reported cases, are hypertelorism, bulbous nasal tip, thin upper lip and large mouth. Gross and fine motor delay and mild to moderatre mental retardation are common.

In contrast, terminal 7q deletion, inclusive of or distal to band 7q33, results in a severe syndrome of physical anomalies and mental retardation including growth retardation, microcephaly, and ocular abnormalities. Mild holoprosencephaly may be present.

The clinical features of our two cases, together with the few reported cases of 7q33 deletion, begin to define a new chromosomal deletion syndrome of mild to moderate mental retardation and a few, but highly characteristic physical abnormalities. This is in contrast to the severe anomalies associated with more distal long arm deletions. The scarcity of reports of this deletion may be due to the combination of relatively mild clinical features and a subtle interstitial deletion. Awareness of this chromosomal lesion will likely result in the ascertainment of additional cases and a more complete description of the 7q33 deletion syndrome.

Program Nr: 1937 from the 1999 ASHG Annual Meeting

Further delineation of the Van Den Ende-Gupta syndrome of blepharophimosis, joint contractures, arachnodactyly and normal development. *D.N. Schweitzer[1], R.S. Lachman[1], B.D. Pressman[2], J.M. Graham, Jr.[1]*. 1) Medical Genetics Birth Defects, UCLA School of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA; 2) Department of Radiology, Cedars-Sinai Medical Center, Los Angeles, CA.

We describe two Hispanic brothers born to unrelated parents with a unique combination of characteristic dysmorphic features, skeletal abnormalities, normal development and unique cerebellar hyperplasia. This syndrome was previously delineated by Van Den Ende and Gupta in 1992 and 1995. This is the fourth report confirming this autosomal recessive syndrome characterized by blepharophimosis, beaked nose, hypoplastic maxilla, everted lower lip, slender and long hands and feet, arachnodactyly, self-limiting congenital joint contractures, distinctive skeletal findings and normal development. Recurrence risk in our family confirms suspected autosomal recessive inheritance and brings to six the total number of reported cases from five families, two of which are consanguineous. Our brothers have identical clinical features and both have cerebellar hyperplasia, a unique finding not previously reported. Though the mode of inheritance and some of these clinical features, including blepharophimosis, arachnodactyly and congenital joint contractures, resemble those observed in Marden Walker syndrome, the presence of mental retardation, serious brain malformations, microcephaly, failure to thrive and severe joint limitation in Marden Walker syndrome and not in Van Den Ende-Gupta syndrome, supports a clinical distinction between these two autosomal recessive disorders.

Program Nr: 1938 from the 1999 ASHG Annual Meeting

Greig cephalopolysyndactly is associated with a novel GLI3 point mutation. *L.H. Seaver, M.J. Friez, J.W. Longshore, R.E. Stevenson.* Greenwood Genetic Ctr, Greenwood, SC.

Greig cephalopolysyndactly is a rare autosomal dominant developmental disorder characterized by multiple physical deformities of the hands and feet. The most prominent features include pre-axial and post-axial polydactly as well as syndactly. Additional findings may also include macrocephaly, a high prominent forehead, a broad nose, hypertelorism and mild mental retardation. Disruption and haploinsufficiency of the GL13 gene located on chromosome 7p13 have been associated with Greigs' syndrome. GL13 encodes a putative zinc finger transcription factor expressed in a number of tissues including the limbs in early mammalian embryos. In addition to disruption of the GL13 gene, several point mutations have also been found to be responsible for Greig's syndrome. In this report, we present a male patient with Greigs' syndrome with chromosomes that are apparently normal. Direct sequencing of the first 12 exons revealed a novel C1447G (Gly465SArg) point mutation adjacent to a zinc finger domain. Interestingly, the mother of this patient also carries the point mutation without signs of Greigs' syndrome. Futhermore, the mother has a daughter with a unilateral bifid thumb in which the point mutation is stongly suspected. If confirmed in the daughter, this GL13 mutation would demonstrate a highly variable phenotype.

Program Nr: 1939 from the 1999 ASHG Annual Meeting

Mental retardation and phenotypic features resembling distal arthrogryposis type II-E in a patient with 47,XXX,del(2)(q37.3)qs. *G.S. Sekhon[1], M.S. Williams[2], P.J. Levonian[2], M. Sharifi[1], K.J. Thompson[1]*. 1) Div Clinical Gen, Waisman Ctr, Univ Wisconsin, Madison, WI; 2) Dept of Pediatrics, Gundersen Lutheran Medical Ctr, La Crosse, WI.

We report a 39 year old female with congenital contractures of the fingers and toes, distal digital hypoplasia and pseudoclubbing of the fingers and thumbs, limitation of motion of the temporomandibular joint, scoliosis, microcephaly and moderate mental retardation. A clinical diagnosis of Distal Arthrogryposis Type II-E was assigned.

Initial unbanded chromosomes (1961) showed a karyotype of 47,XXX. High resolution chromosomes were performed in 1998. 47,XXX was confirmed. In addition, one copy of chromosome 2 had a satellite attached to the distal end of the long arm. Multiple banding techniques were applied together with fluorescence in-situ hybridization (FISH) studies to determine the nature and origin of the additional material. NOR-staining detected a positive region on the long arm of chromosome 2. FISH studies with 2q37 and 2q37.3 probes were performed to rule out a deletion of the long arm of chromosome 2. Two signals were obtained with the 2q37 probe, however, only the non-satellited chromosome 2 homologue had a signal with the 2q37.3 probe.

The patient's clinical features have never been reported in any female with triple X. Mental retardation has been reported in patients with 2q37 deletions. There is a single case report of a patient with 2q35 deletion with finger flexion contractures, but neither this patient nor any reported patients with 2q37 deletion have had distal digital hypoplasia. Our patient does not have clinical features of Albright's hereditary osteodystrophy, which has been reported in some patients with 2q37 deletion. Whether our patient's features are due to the 2q37 deletion, are due to an interaction between the chromosomal deletion and gene overdosage from the triple X, or has 3 distinct genetic conditions is indeterminate at this time.

Program Nr: 1940 from the 1999 ASHG Annual Meeting

Incidence of autism and attention deficit hyperactivity disorder (ADHD) in individuals with deletions of chromosome 18q. *M. Semrud-Clikeman[1], J.D. Cody[2], C.I. Kaye[2], R.J. Leach[2,3].* 1) Department of Educational Psychology, University of Texas, Austin, TX; 2) Department of Pediatrics, UT Health Science Center, San Antonio, TX; 3) Department of Cellular and Structural Biology, UT Health Science Center, San Antonio, TX.

We assessed 46 children (>3yrs old) with deletions of 18q. Six were found to qualify for a diagnosis of autism using the Children's Autism Rating Scale. Autism is a rare disorder occuring in approximately 1-2% of the population. The behavior of these children is characterized by lack of interest in relating to others, stereotypy, and language skill delays, frequently beginning between ages 2 and 3. We found an incidence of autism of 13%, higher than would be expected by chance. There was no difference between the autistic and nonautistic groups on performance IQ (mean 58 and 67.4 respecively). We also identified several children (5 of the remaining 40) with autistic-like behaviors who do not qualify for a diagnosis of autism. There is emerging data from other investigators who are studying autism that there may a predisposition gene for autism on chromosome 18q. Case studies of children with deletions of 18q have found evidence for behavioral difficulties including ADHD and emotional dyscontrol. ADHD is reported to be found in approximately 3-5% of the general population. We found an incidence of ADHD of 24% which is significantly above expectations. There was no difference between performance IQ of the ADHD and non ADHD samples (mean 59.5 and 67.4 respectively). We have begun assessing psychopathology in these individuals. Out of the 14 children assessed, 9 have shown significant difficulties with emotional dyscontrol, 3 of them with such magnitude that neuroleptic medications were required. These results suggest a vulnerability for emotional and behavior dyscontrol associated with deletions of chromosome 18q. Such vulnerability appears to increase with age and may be associated with puberty. The co-occurrence of autism, ADHD, and emotional dyscontrol syndromes associated with individuals with deletions of 18q speaks to the need to seek interventions beyond traditional medical and educational methods.

Program Nr: 1941 from the 1999 ASHG Annual Meeting

Discordant MZ twins in familial amyloid neuropathy (FAP)-TTRMet30. *J. Sequeiros[1], J.L. Pedrosa[2], T. Coelho[3], L. Gusmão[4], R. Seruca[4], A. Amorim[4], M. Munar-Qués[5]*. 1) UnIGENe-IBMC and ICBAS, Univ Porto; 2) Hosp Oliveira Azeméis; 3) Centro Estudos Paramiloidose, Porto; 4) IPATIMUP, Univ Porto, Portugal; 5) Grupo Estudio PAF, Palma Mallorca, Spain.

FAP-I is an autosomal dominant systemic amyloidosis presenting mainly as a peripheral neuropathy. The most common defect is a Met30 substitution in transthyretin (TTR); there is a large variability in age-at-onset (mean: 56.7 yrs in Sweden; 46.7 in Majorca; and 33.5 in Portugal, 35.6 in Japan and 32.4 in Brazil.

In a set of Majorcan twins, J begun FAP-I at age 38 and G at age 50 (2 yrs after J died); G was confined to a wheelchair 7 yrs after, while J was still walking with 2 canes when he died (12 years after onset); only G had severe amyloid deposits of the vitreum (bilateral vitrectomy); J died at age 48 and G at 63; we used old paraffin blocks to extract DNA; L(MZ) was 0.9903. In a set of Portuguese MZ twins, there was the simultaneous occurrence of sacrococcygeal cysts, contrasting with relative discordance for FAP: A had onset at age 30 and J at 34; initial symptoms were a severe sensorimotor syndrome with early involvement of the arms, in A, while J had severe weight loss; A also had cardiac rhythm disturbances, orthostatic hypotension, diarrhea and ulcers in both feet, but J had only a light sensorimotor syndrome and gastrectasia; A died upon many complications after liver transplant, at age 34; J had a successful transplant at 35; DNA was extracted from necropsy material in A; L(MZ) was 0.9999.

Two other sets of twins are known. Significant discordance is present in all four pairs (3 male), both in clinical severity and age-at-onset (up to 12 yrs). The TTR mutation and its genetic modifiers are not the only determinants of age-dependant penetrance and variable expressivity in FAP: other, non-genetic, factors (undetected environmental factors or stochastic events at the molecular and/or cellular level, before or during the twinning process) must be operating. Different patterns of somatic mosaicism for (nuclear) modifying genes or different distributions of mithochondrial modifiers could explain these different expressions.

Program Nr: 1942 from the 1999 ASHG Annual Meeting

Proteus syndrome with infantile myofibromatosis and severely disturbed adipose growth and differentiation. *A.L. Shanske[1], H. Kang[1], R.J. Shprintzen[2], J. Goodrich[1], R. Marion[1]*. 1) Ctr Congenital Disorders, Montefiore Medical Ctr, Bronx, NY; 2) Communications Disorders Unit, State University of New York Health Sciences Center, Syracuse, NY.

Proteus syndrome was first described in 1979 as a complex hamartomatous disorder including partial gigantism and asymmetry of the limbs and various neoplasms. The most commonly observed tumors have been subcutaneous hemangiomas, lymphangiomas and lipomas. The overgrowth and neoplastic changes observed in these patients are thought to result from mosaicism for a dominant lethal gene. We have had the opportunity to evaluate a 7 year old girl with this disorder with an unusual course including a previously unreported neoplasm.

JM was the 8 pound product of a term pregnancy. Two large masses were excised at 2 weeks of age from her chest and abdomen. The histology was consistent with infantile myofibromatosis. Facial asymmetry was apparent by 2 months of age and she developed a partial complex seizure disorder at 4 months. Imaging studies showed a right posterior abnormality consistent with cerebral dysplasia or a prenatal infarct with an associated volume loss. The facial asymmetry progressed to hemiatrophy of the right calvarium down to the right lower jaw. She underwent craniofacial reconstruction at one year with multiple osteotomies and recontouring of the orbital rim and correction of orbital dystopia. She has also had strabismus repair and repair of a left cavovarus foot deformity. Her physical examination at the present time reveals a number of additional significant findings including obesity, overgrowth of the right foot, port-wine stain of the right cheek and chest, acanthosis nigricans, and lipodystrophy of the back.

Our patient fulfills the recently published consensus diagnostic criteria for the Proteus syndrome (Am J Med Genet 84:389-95, 1999). In addition, she has had a rare pediatric tumor which has not been previously reported in association with Proteus syndrome and more extensive signs of disturbed adipose growth and differentiation including decreased subcutaneous tissue, obesity and acanthosis nigricans.

Program Nr: 1943 from the 1999 ASHG Annual Meeting

PCR-SSCP analysis of FMR1 gene in autistic and mentally retarded children in Japan. *K. Shinahara, T. Saijo, K. Mori, Y. Kuroda.* Department of Pediatrics, School of Medicine, University of Tokushima, Tokushima, Japan.

Fragile X syndrome is characterized by long face, prominent chin, large floppy ears, macro-orchidism, retarded language development, hyperactivity and seizure, and is one of the most common cause of mental retardation. However, the craniofacial features are difficult to identify in some patients. It is also reported that some patients with fragile X syndrome show autistic features although the relationship between fragile X syndrome and autism is controversial. The majority of the patients with fragile X syndrome has an expansion of trinucleotide (CGG) repeat in the 5 sequence of FMR1 gene, but a partial or total deletion of the gene has been found in a couple of patients and one nucleotide substitution in the coding region in a patient. In order to study the frequency of the FMR1 mutation among mentally retarded and/or autistic patients, we performed polymerase chain reaction (PCR)-single strand conformation polymorphism (SSCP) analysis of the gene. Among 50 patients analyzed (45 males and 5 females, including two pairs of siblings), abnormal SSCP pattern was observed in two samples derived from two unrelated female patients; exon 8 from patient A and exon 9 from patient B. When exon 8 from patient A was cloned and sequenced, a G to A substitution at nucleotide 662, which alters Glu at 221 to Gly, was observed in four clones out of six, whereas the remaining two clones had the normal sequence. When 17 clones carrying exon 9 from patient B were sequenced, 11 clones had an A to C substitution at nucleotide 879. It caused splicing error, presumably resulting in reduced amount of normal FMR1 protein. No mutation was found among boys. Although uncommon, point mutation in FMR1 gene may be the cause of mental retardation in Japanese population.

Program Nr: 1944 from the 1999 ASHG Annual Meeting

Clinical and molecular exploration of two families with demyelinating neuropathies and non neuropathic features. *S. Sigaudy[1], P. Nègre[1], B. Michel[2], J.F Mattéi[1], N. Philip[1,3], N. Lévy[1,3].* 1) Département de Génétique Médicale, Hôpital d'enfants de la Timone, Marseille, France; 2) Service de Neurologie, Hôpital Sainte-Marguerite, Marseille, France; 3) InsermU491 "Génétique Médicale, et Développement", Faculté de Médecine, Marseille, France.

We report two families with affected individuals presenting a demyelinating peripheral neuropathy with low Nerve Conduction Velocities (NCVs). In one, originating North-Africa, 2 brothers and 2 sisters were affected were initially diagnosed as Charcot-Marie-Tooth type 1. One male and 1 female presented a severe phenotype with foot deformity and distal muscular atrophy for both upper and lower limbs. Two other affected individuals, had milder neurological findings. All affected members presented profund deafness and mild mental retardation. A possible asymptomatism in parents, coupled to the absence of consanguinity in this family, made uncertain the mode of inheritance. We present a loci exclusion for PMP22, Cx32 and Po in this family. Connexin 26 mutations were also not found. In another family, two brothers were affected with demyelinating neuropathy, deafness, and chronic nephritis. This nephropathy was classified as Alport syndrome. Neurological features started in childhood, and at 13 and 15 years old respectively, they presented a severe neuropathy with walking difficulties, pes cavus and distal muscle atrophy predominating to the hands. Electrophysiology revealed a denervation process with low NCVs at all limbs. Audiograms determined the sensorineural hearing loss and showed the high tone frequencies beeing predominantly affected. Terminal renal failure leaded to transplantation. The other siblings and the parents as well, were healthy without biological or clinical mild disorders making it impossible to suggest a possible recessive autosomal or X-linked inheritance mode although the parents were native from the same village in Morocco. In this family, we'll present the molecular explorations performed to date which leaded to exclude main loci usually involved in CMT1. Further explorations are now in process for the already known loci beeing linked to recessive forms of hereditary demyelinating neuropathies.

Program Nr: 1945 from the 1999 ASHG Annual Meeting

A severe case of Trichothiodystrophy due to a DNA repair defect. *J. Sigmundsson[1], L. Woolf[1], E. Magenis[1], R.D. Steiner[1], J. Roberts[2], D. Pillers[1], R. Nixon[1], A.N. D'Agostion[1], D. Wilson[3], P. Stenzel[1], A. Lehmann[4].* 1) Oregon Health Sciences University, Portland; 2) Portland; 3) Casey Eye Institute, Portland; 4) MRC Cell Mutation Unit, Sussex University, Falmer, Brighton, U.K.

The patient was a male, born at 35 weeks gestation. Birth weight was 2.1 kg. Pregnancy was complicated by severe pregnancy induced hypertension. Amniocentesis had revealed normal chromosomes. Family history was negative. Sparse, dry, brittle scalp hair was noted and the patient had mild edema at birth that resolved in a few days, but left peeling skin, shiny in parts and taut in the hands. The skin texture normalized in the first few weeks. Most of the hair fell off in the first few weeks of life. The patient had feeding difficulties and failure to thrive. Hair studies revealed low sulfur content and TTD-type hair. At three months of age the patient was noted to have subtle facial asymmetry and low muscle tone. The patient expired suddenly at three months. He had never been exposed to sunlight. Complete autopsy revealed congenital cataract, focal perimedullary fibrosis of the caudal spinal cord and pulmonary parenchyme hemorrhage.

DNA repair studies on skin fibroblasts that had been obtained earlier showed abnormal response to UV irradiation similar to those reported for other TTD patients. Nucleotide excision repair, as measured by unscheduled DNA synthesis was 15-20% of that in normal cells, and RNA synthesis failed to recover after UV irradiation, as previously reported in cells from patients with TTD and other repair-deficient disorders.

This report emphasizes the importance of early awareness of this disorder and prompt work up and demonstrates that not all patients with TTD that have a DNA repair defect have a history of sun sensitivity. More than 50% of patients with TTD, most of whom have had sun sensitivity, have been found to have a DNA repair defect, most of them having mutations in the xeroderma pigmentosum, complementation group D (*XPD*) gene and, in one affected family, in the *XPB* gene. Complementation studies on our patient are currently in hand.

Program Nr: 1946 from the 1999 ASHG Annual Meeting

Clinical and cytogenetic findings in three new cases of deletion 10q26. *S. Sigurdardottir[1], M.T. Geraghty[2], V. Praphanphoj[2], G.H. Thomas[1,2], B.K. Goodman[2].* 1) Kennedy Krieger Institute, Baltimore, MD; 2) Inst of Genetic Medicine, Johns Hopkins Univ School of Medicine, Baltimore, MD.

Deletions of the distal long arm of chromosome 10 [10q26-qter] are rare, with only 16 reported patients. Consistent clinical findings are psychomotor retardation and typical craniofacial features, including prominent nasal bridge, strabismus and hypertelorism. Significant growth retardation and major anomalies, most often involving the heart and urogenital system, have been reported in some patients. We report on 3 patients with de novo 10q26 deletions. All three were born at term with normal birth weights. The first patient, a male, had severe developmental delay, VSD, hydronephrosis, patent urachus and bilateral sensorineural hearing loss caused by a Mondini malformation. At age 3 years he was growth retarded, microcephalic with low set abnormal ears, and hypotonia with decreased muscle strength. Facial features included esotropia, pointed chin, and prominent nasal bridge. Peripheral blood karyotype was 46, XY, del (10) (q26.12). In contrast, the second and third patients had a more distal deletion [del (10) (q26.2)] associated with less severe developmental delay and without major malformations. The second patient, a 3 year old girl, had growth delay, microcephaly with frontal bossing, widow's peak, prominent nasal bridge and bilateral fifth finger clinodactyly. She had a previous normal karyotype. The deletion in this case was confirmed using a 10q-specific subtelomeric probe. The third patient, a 14-month-old girl, was the most mildly affected. Her head circumference and weight were normal but her height was below the 5th centile. She had esotropia, prominent nasal bridge, tapering fingers and axial hypotonia. These 3 cases support the suggestion that patients with distal 10q deletions are not a uniform group. Contrary to some previous reports, we found a relationship between the size of the deletion and severity of clinical findings. The finding of a Mondini malformation in the first patient is interesting, as sensorineural hearing loss has previously been reported in three patients with deletions in this area. Further molecular cytogenetic studies are in progress.

Program Nr: 1947 from the 1999 ASHG Annual Meeting

Prolonged survival in a case of atelosteogenesis type 2. *V.M. Siu[1,2], B.A. Gordon[1], Y. Ouellette[2], A. Superti-Furga[3].* 1) Medical Genetics Program of Southwestern Ontario and CPRI; 2) Children's Hospital of Western Ontario, London, Ontario, Canada; 3) University Children's Hospital, Zurich, Switzerland.

Diastrophic dysplasia (DTD), atelosteogenesis type 2 (AO2), and achondrogenesis 1B (ACG1B) form a spectrum of autosomal recessive chondrodysplasias caused by mutations in the diastrophic dysplasia sulfate transporter (DTDST) gene. Though similar in histologic and ultrastructural features, clinical severity varies widely. A male infant was born at 38 weeks to a nonconsanguineous couple. Ultrasound at 30 weeks had documented hitchhiker thumbs suggestive of DTD. At birth there was severe limb shortening with nubbin-like digits, in addition to edema, macrocephaly, cystic ears, hypertelorism, short neck, cleft palate, small chest, and wide gap between halluces and second toes. Radiographic investigation documented cervical kyphosis, thoracic scoliosis, lumbar lordosis with horizontal sacrum, V-shaped distal humeri, dislocated elbows, bowed radii and tibiae, and dumbbell-shaped femora. The phenotype was compatible with AO2; limb shortening was almost as severe as in ACG1B. MRI scan has revealed cerebral atrophy and BAERA has shown bilateral hearing loss. Molecular analysis confirms compound heterozygosity for the common Finnish mutation and a Q2021C substitution in the cytoplasmic tail of the DTDST gene, each mutation being present in one parent. Urinary glycosaminoglycans were grossly undersulfated. The baby was highly unstable and ventilator dependent in the first year of life due to pulmonary hypoplasia and severe tracheobronchomalacia. At 13 months of age, he breathes independently, is visually alert but makes no sounds, requires gastrostomy feeds, is unable to sit, and has no movement of his digits. Among patients with DTDST skeletal dysplasia surviving beyond the neonatal period, this baby is the most severely affected reported thus far, in terms of limb findings. Despite poor prognosis, parents advocated strongly for life-supporting measures. Mechanical ventilation and repeated resuscitations can allow survival even in the presence of severe skeletal dysplasia. We are concerned about long-term outcome and and anticipate complications related to the cervical kyphosis.

Program Nr: 1948 from the 1999 ASHG Annual Meeting

Pena-Shokeir phenotype secondary to neonatal neuroaxonal dystrophy. *R. Smith[1], J.A. Golden[2], S. Saitta[1], A.D. Kline[3], J.E. Ming[1], D.M. McDonald-McGinn[1], D.F. Carpentieri[2], B.W. Little[4], L.B. Rorke[2], E.H. Zackai[1].* 1) Division of Human Genetics and Molecular Biology and; 2) Department of Pathology, The Children's Hospital of Philadelphia, Philadelphia, PA; 3) Division of Clinical Genetics, Department of Pediatrics, Sinai Hospital of Baltimore, Baltimore, MD; 4) Department of Pathology and Laboratory Medicine, Allegheny University of the Health Sciences, Philadelphia, PA.

Infantile neuroaxonal dystrophy, also known as Seitelburger's disease, is a rare autosomal recessive neurodegenerative disease. The onset of clinical disease classically begins during the second year of life with a progressive loss of developmental milestones. Although presentation by 6-9 months is not uncommon, neonatal onset is not generally recognized. Pathologically infantile neuroaxonal dystrophy is characterized by spheroids in central and peripheral nerve axons. We present two unrelated male sibling pairs, who presented with the fetal hypokinesia/akinesia sequence, other minor and major anomalies, and neonatal demise. Neuropathological examination demonstrated axonal spheroid pathology in all four males consistent with a form of neuroaxonal dystrophy. The distribution of spheroids in the first sibling pair was primarily in the dentate nucleus, globus pallidus and thalamus. These brothers also demonstrated cerebellar dysplasia, inferior frontal polymicrogyria and ventriculomegaly. In the second sibling pair axonal spheroids were predominantly found in the cerebral white matter and in the basis pontes. These two sibling pairs, along with a few other reported cases, expand the clinical spectrum of neuroaxonal dystrophy to include a neonatal onset form. These cases provide additional support for autosomal or X-linked recessive inheritance in neonatal neuroaxonal dystrophy, and demonstrate that neonatal neuroaxonal dystrophy must be considered as a neuropathological etiology of the fetal hypokinesia sequence.

Program Nr: 1949 from the 1999 ASHG Annual Meeting

Radiographic diagnosis of Shwachman-Diamond Syndrome in a neonate. *W. Smith, A. Amalfitano, C. Miller, A. Patterson.* Divisions of Medical Genetics and Pediatric Radiology, Duke University Medical Center, Durham, NC.

Shwachman-Diamond syndrome (SDS) is a rare, autosomal recessive disorder characterized by pancreatic insufficiency, bone marrow dysfunction and skeletal dysplasia. Diagnosis is usually made in childhood secondary to pancreatic or blood disorders. Restrictive thoracic dystrophy (RTD) in the neonatal period has been retrospectively reported in several patients with SDS. We report a neonate with RTD who was subsequently diagnosed with SDS. This African American male was the 2.6kg (25th%) product of a normal 38 week pregnancy to a G10P5 mother; birth length 45cm (10th%), OFC 33.5cm (50-75th%). He had symmetric rhizomelic limb shortening and a very narrow, bell shaped chest: inter-nipple distance 6.3cm (<3rd %), chest circumference 28cm (3-50th %). Radiographs obtained at age 6 days demonstrated markedly shortened AP and transverse chest diameters with small, short, anteriorly cupped ribs. The thoracic and lumbar vertebrae were ovoid with end-plate sclerosis. There was significant metaphyseal dysplasia of the long bones with deep cupping but no irregularity. A tentative diagnosis of SDS was made based solely on the skeletal findings. Subsequent investigations revealed low serum trypsin and increased stool fat consistent with pancreatic insufficiency. By five weeks of age he developed recurrent vomiting and diarrhea, failure to thrive and a significant anemia: hgb 4.8 mg/dl, hct 15%. Physical examination demonstrated hepatosplenomegaly. A midgut nonrotation was diagnosed and he underwent surgical repair. Simultaneous liver biopsy showed extensive macrovesicular steatosis with canalicular cholestasis and abundant iron. Postoperatively he remained ventilator and blood product dependent. Due to neutropenia he developed concurrent fungal, coagulase negative staphylococcal, and enterococcal sepsis and died at age 10 weeks. Post-mortem histology of the pancreas was characteristic of SDS with highly vacuolated lobules, atrophic acinar spaces and preserved islets. This patient, in whom a prospective radiologic diagnosis of SDS was made as a neonate, suggests that disease severity may be positively correlated with the radiographic features and the degree of RTD.

Program Nr: 1950 from the 1999 ASHG Annual Meeting

Distichiasis, lymphedema, and cleft palate (DLC) : a discrete Mendelian entity with autosomal dominant inheritance. *V. Soupre[1], C. Houdayer[2], M.-P. Vazquez[1], M. Bahuau[1,2]*. 1) Chirurgie Maxillo-Faciale et Plastique et Stomatologie, Hôpital d'Enfants Armand-Trouseau, Paris, France; 2) Biochimie et Biologie Moléculaire, Hôpital d'Enfants Armand-Trouseau, Paris, France (biochimie.trousseau@trs.ap-hop-paris.fr).

In view of identifying monogenic pedigrees with syndromic cleft palate, we have investigated 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, and cleft palate/velum in two, without the previously reported association to the Pierre Robin anomalad. Other ophthalmologic anomalies included divergent strabismus and early-onset myopia. Pterygium colli, congenital heart disease or facial dysmorphism were not features here. This observation confirms previous singular or familial reports with this rare cluster of malformations. We believe this familial condition is a discrete Mendelian trait distinct from previously delineated entities such as distichiasis (MIM 126300), distichiasis-lymphedema (MIM 153400), type-1 (Nonne-Milroy's; MIM 153100), or type-2 (Meige's; MIM 153200) hereditary lymphedema. Linkage analysis in this and other like kindreds is warranted to map the underlying genetic defect, test for allelism to germane but phenotypically distinct entities, and eventually provide new insights into the developmental/signaling pathways involved in palatal closure.

Program Nr: 1951 from the 1999 ASHG Annual Meeting

Clinical study and use of FISH analysis to diagnostic approach of Brazilian patients with Williams-Beurens syndrome. *S. Sugayama[1], M. Chauffaille[2], K. Abe[2], D. Bertola[1], L. Albano[1], C. Utagawa[1], A. Catelani[2], M. Obelar[2], N. Kusagari[2], P. Perroud[2], C. Kim[1]*. 1) Pediatrics, Instituto da Crianca, Sao Paulo, Brazil; 2) Fleury Laboratory, Sao Paulo, Brazil.

Williams-Beurens syndrome (WBS) is a rare (1/20,000-1,50,000 livebirths) neurodevelopmental disorder affecting multiple systems.The main features are typical elfin facies, heart defects, mental deficiency, friendly personality and hypercalcemia in infancy.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. We report on clinical evaluation and FISH analysis(VYSIS/LSI-Williams Syndrome Region DNA FISH probe)from 21 patients (12M/9F);all cases were sporadic. The mean age at diagnosis was 5,2 years (2mo-12y) and the mean current age is 9,7 years (23mo-17,5y).All patients presented typical elfin facies, developmental delay/mental retardation, friendly personality,skeletal and dental anomalies;fourteen (67%) had ocular anomalies, specially strabismus; thirteen (62%)had congenital cardiac defect:eight of them had supravalvar aortic stenosis (SVAS); thirteen (62%) had short stature; ten (48%) presented transitory hypercalcemia; seven (33%) had inguinal hernia; five (24%) had renal anomalies and 3 boys had genital anomalies.One girl presented precocious puberty at 5 years of age.She is likely the second case of WBS patient with this disorder in the literature. Deletion was found in seventeen (81%)patients. FISH analysis of the elastin locus is a useful test as an initial diagnostic approach for WBS. However, the presence of two copies of the elastin locus in a patient does not rule out WBS syndrome.Our four patients without deletion presented typical facies and behavioral but no cardiac defects.Therefore, these results support the importance of molecular studies in further delineation of the WBS phenotype.

Program Nr: 1952 from the 1999 ASHG Annual Meeting

De novo chromosome 2 pericentric inversion in a stillborn infant with Cerebro-Costo-Mandibular syndrome.

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Cerebro-Costo-Mandibular syndrome (CCMS) is a rare condition that consists of severe micrognathia, rib anomalies, cleft palate, and in some cases cerebral anomalies. There are at least 50 patients with CCMS in the literature. Infants with CCMS have a greater than 50% mortality in the first year of life because of lung hypoplasia and upper airway obstruction due to micrognathia and glossoptosis. Although mostly sporadic, pedigrees consistent with autosomal recessive and autosomal dominant inheritance have been documented. Mechanisms that would account for most reported cases include autosomal dominant inheritance with reduced penetrance, germline mosaicism, and genetic heterogeneity. All reported karyotypes are normal and no single putative gene has been implicated.

A 23 year old, $G_3P_1A_1$, African American female presented at 18 weeks gestation for evaluation of a fetal thickened nuchal fold. Ultrasound revealed scalp edema, severe micrognathia, small chest with no identifiable ribs, and possible bifid left thumb. Amniocentesis revealed a fetal karyotype of 46,XX,inv(2)(p24.2q33) and parental karyotypes were normal. Intrauterine fetal demise occurred at 26 weeks and a diagnosis of CCMS was made at post mortem examination. Physical findings in addition to those seen prenatally included broad alveolar ridges, lowset ears with bilateral imperforate auditory meatus, short sternum with bell shaped chest, labia majora hypoplasia, elbow pterygium, proximally placed thumbs, ulnar deviation of the hands, left preaxial polydactyly, and rocker bottom feet.

This case is significant because it is the first case of CCMS with a documented cytogenetic abnormality. This suggests that: (1) a gene or contiguous genes exist at 2p24.2 or 2q33 that may be important in fetal development of ribs and mandible; and (2) it suggests that an autosomal dominant gene or contiguous gene deletion may explain the etiology of CCMS.

Program Nr: 1953 from the 1999 ASHG Annual Meeting

Transverse Limb Defects Associated with Vascular Abnormalities: Atypical Presentation of Adams-Oliver Syndrome? *A.S. Teebi[1], N. Al-Sannaa[1], I. Adatia[2].* 1) Pediatrics & Genetics, Hosp Sick Children, Toronto, ON, Canada; 2) Cardiology & Critical Care Medicine, Hospital for Sick Children, Toronto, ON, Canada.

Adams-Oliver syndrome (AOS) is a well-recognized entity that associates cutis aplasia congenita with terminal transverse limb defects and other anomalies. Poland sequence has been considered part of a spectrum having the same pathogenesis. Wide intrafamilial and interfamilial variability was noted. Over the last decade, there has been a surge of publications documenting the association of vascular and cardiac anomalies in AOS. It was estimated that congenital cardiac malformations are present in about 20% of all AOS patients. If the association of the other vascular anomalies were considered, it would appear that this group of anomalies represents an important component of the syndrome. We report a 4 years old boy with terminal transverse limb defects associated with primitive persistence of primitive aorto-pulmonary vascular connections leading to supra-systemic pulmonary artery pressure. This patient did not display the other manifestation of AOS like cutis aplasia congenita or Poland sequence and the family history is negative for that type of anomalies. Based on the association of vascular anomalies as an emerging component of Adams-Oliver Syndrome, we suggest that this patient is an example of AOS with atypical presentation.

Program Nr: 1954 from the 1999 ASHG Annual Meeting

A rare 12p distal interstitial deletion with radial ray anomaly of the hands and ectrodactyly of the feet: Cytogenetic and molecular delineation. *L.L. Thomson[1], C.K. Stein[1,2], A.E. Shrimpton[2], A.M. Willey[3], J.J. Hoo[1]*. 1) Pediatrics, State Univ. NY Health Sci Ctr, Syracuse, NY; 2) Dept. Pathology, State Univ. NY Health Sci Ctr, Syracuse, NY; 3) NY State Dept. Health, Albany, NY.

12p deletions are rare, and of the reported cases, most are proximal interstitial deletions. Only one previous case (Romain et al., 1987) showed a distal interstitial deletion of 12p. While the cases with proximal deletion show severe mental retardation, the distal deletion case of Romain et al. was associated with mild mental retardation.

Our patient was born with normal birth weight, length, and head circumference. The following dysmorphic features were noted at birth: left iridal coloboma, bilateral posteriorly rotated ears, grade 2 hypospadias, bilateral hypoplastic thumbs, and bilateral ectrodactyly of the feet. At almost 2 months of age, he was alert and starting to smile. Muscle tone was normal. No internal organ abnormality was noted. The high resolution karyotype revealed a distal interstitial deletion of the short arm of chromosome 12 [46,XY,del(12)(p13.1p13.2) de novo]. FISH utilizing a whole chromosome paint probe for 12 showed signal along the entire length of both 12s, but no evidence of signal elsewhere in the cells. Molecular studies showed the deleted 12 to be of paternal origin. DNA probes in distal p12.3 were deleted as well as the most distal probe in 13.2, refining the breakpoints to12p12.3p13.2.

Like the patient of Romain et al., our case may only have mild mental retardation. However the radial ray anomaly of the hands and the ectrodactyly of the feet have not been reported in any previous 12p deletion cases. The molecular delineation of the breakpoints may be useful for future mapping of a gene causing the anomaly in the distal extremities.

Program Nr: 1955 from the 1999 ASHG Annual Meeting

Premature osteoporosis in neurofibromatosis type 1. *C.J. Tifft, L.L. Tosi, P.H. White, K.N. Rosenbaum.* Children's National Medical Center, Washington, DC.

Neurofibromatosis type 1 (NF1) has been associated with skeletal manifestations including scoliosis, pseudoarthrosis and sphenoid wing dysplasia. A small number of patients have also been reported with osteomalacia. We report an adolescent and young adult with generalized osteoporosis, a finding not previously associated with NF1. Case 1 is a 12-year-old female first seen at 9 years of age for evaluation of possible NF1. The diagnosis was confirmed by NIH consensus criteria by the presence of Lisch nodules, axillary and inguinal freckling, multiple cafe-au-lait spots, and cutaneous neurofibromas. Fluorescence in situ hybridization demonstrated a deletion of 17q11.2. Additional problems included hydrocephalus secondary to aqueductal stenosis requiring shunt placement and bilateral pes planus requiring surgical intervention. At 11 years of age, during a period of limited activity, this child experienced back pain and radiographs showed vertebral compression fractures. Bone density was evaluated by DEXA scan revealing severe osteoporosis (T-score of -4.79 at L1 to L4, -4.68 at the femoral neck, and -4.51 at the left hip). The patient has been maintained on calcitonin, calcium and vitamin D daily. Case 2 is a 27 year old female diagnosed with NF1 at 12 years of age based on multiple cafe-au-lait spots, cutaneous and plexiform neurofibromas, Lisch nodules and scoliosis. At 25 years of age a follow-up spine radiograph showed compression fractures of T6 and T7. A DEXA scan showed moderately severe osteoporosis (T-score -2.49 for the lumbar spine and -2.17 at the femoral neck). A bone biopsy revealed decreased bone turnover and excluded osteomalacia. A CBC and serum chemistries including calcium and phosphorus were normal as were 24-hour urinary excretion of calcium, phosphorus and creatinine. The patient was placed on bisphosphonates and began a regular program of weight-bearing exercise. A one-year follow-up study showed an increase in bone density. Osteoporosis may be a more common finding in children and young adults with NF1 than previously recognized, particularly those with skeletal involvement or inactivity. DEXA scanning in a subgroup of potentially at-risk patients may therefore be appropriate.

Program Nr: 1956 from the 1999 ASHG Annual Meeting

Study of genetic factors contributing to both isolated and MCA cases of omphalocele. *C.P. Torfs[1], E.V. Semina[2], M. Knipp[2], F. Lorey[4], J.C. Murray[2,3].* 1) CA Birth Defects Monitoring, Emeryville, CA; 2) Department of Pediatrics, Univ Iowa, Iowa City, IA; 3) Department of Biol Sciences, Univ Iowa, Iowa City, IA; 4) Genetic Disease Branch, DHS, Berkeley, CA.

We have studied all cases of omphalocele born in the area of catchment of the California Birth Defects Monitoring Program between 1983 and 1994, excluding trisomies, conjoined twins, and cases with a known genetic or chromosomal defect such as Beckwith-Wiedemann syndrome. For 216 cases, newborn screening blood spots were available from the Genetic Disease Branch of California. The group consisted of 92 (43%) infants with an isolated defect and 124 with multiple congenital anomalies (MCA) including 6 with a recognized syndrome and one mosaic XXX. 59% of the isolated cases and 48% of the MCA cases were male. There was no significant difference in maternal age between the two groups. Maternal ethnicity was 29 % Hispanic, 50 % non-Hispanic Caucasian ;10% African American , and 6 % Asian and 4% Other. To evaluate genetic contributions to both isolated and MCA cases, we screened for mutations in genes known to predispose to omphalocele in specific syndromes (PITX2 in Rieger syndrome, GPC3 in Simpson-Golabi-Behemel syndrome) or to be strongly expressed in the abdominal region, including PITX1. The entire coding regions and all the exon-intron junctions of PITX2 and PITX1 were screened in 208 cases by SSCP analysis and no mutations were identified. Screening for the GPC3 is ongoing. This study provides an opportunity to evaluate an unbiased collection of omphalocele cases and to determine the effect of specific gene mutations on etiology.

Program Nr: 1957 from the 1999 ASHG Annual Meeting

Multisystem involvement in congenital insensitivity to pain with anhidrosis (CIPA), a nerve growth factor receptor (TrkA) related disorder. *E. Toscano[1], R. Della Casa[1], S. Mardy[2], G. Marzano[1], N. Varricchione[1], F. Sadile[1], C. Pignata[1], Y. Indo[2], G. Andria[1]*. 1) Pediatrics, "Federico II" University, Naples, Italy; 2) Pediatrics, Kumamoto University,Honjo, Kumamoto, Japan.

Congenital insensitivity to pain with anhidrosis (CIPA) is an autosomal recessive sensory and autonomic neuropathy. CIPA is caused by a defect of the high affinity tyrosine kinase (TrkA) receptor for nerve growth factor (NGF). PC,female, age 12 years and MC, female, age 5 years, are two unrelated CIPA patients, diagnosed on the basis of insensitivity to pain, self mutilating behavior, recurrent episodes of fever, absence of sweating and mental retardation, with presence of fungiform papillae and corneal reflexes and normal structure of sweat gland at skin biopsy. Both patients presented bone involvement (joint deformities, pelvic dysplasia, frequent fractures) and immune system alterations (decreasing of proliferative response of peripheral blood mononuclear cells to PHA, Con-A and CD3X-L stimuli). PC died from renal insufficiency, secondary to a rapidly progressive systemic amyloidosis. Molecular analysis of the TrkA gene revealed in PC a homozygous G to A transition, causing a Gly to Ser substitution at amino acid 708, and in M.C. a C to T transition, causing a Gln to a termination codon at amino acid 9, respectively (Mardy et al AJHG 64:1570-79,1999). NGF increases the synthesis of acetylcholine in cholinergic neurons, playing a role in the survival of selected populations of neurons. NGF can also modulate synaptic plasticity and transmission and maintain a balanced interplay between nervous, immune and endocrine systems. Bone involvement in CIPA patients suggests that NGF exerts also a role in skeletal cell metabolism. CIPA is the first human genetic disorder caused by a defect in the neurotrophin signal transduction system and this is the first clinical report about CIPA patients characterized on the molecular ground. The clinical phenotypes of our patients reveal that a multisystem involvement may be observed in CIPA patients. Molecular characterization might improve our understanding of the physiological roles of NGF/TrkA system in human and eventually suggest new therapeutic approaches.

Program Nr: 1958 from the 1999 ASHG Annual Meeting

Neurological phenotype in Waardenburg type 4 patients with SOX10 mutations. *R. Touraine, T. Attié-Bitach, J. Amiel, A. Pelet, J. Auget, A. Munnich, M. Vekemans, S. Lyonnet.* Department of Genetics, INSERM U-393, Hopital Necker-Enfants-Malades, Paris, France.

Waardenburg syndrome type 4 (WS4) or Shah-Waardenburg syndrome is a rare neurocristopathy resulting from the absence of melanocytes and intrinsic ganglion cells of the terminal hindgut. WS4 is inherited as either an autosomal recessive trait due to EDN3 or EDNRB mutations, or an autosomal dominant condition when SOX10 mutations are involved. Here we report on three unrelated WS4 patients with growth retardation and an as yet unreported neurological phenotype with impairment of both the central and autonomous nervous systems and occasionally neonatal hypotonia and arthrogryposis. Each of the three patients was heterozygous for a SOX10 truncating mutation (Y313X in two patients, S351X in one patient). A likely hypothesis for the extended clinical spectrum would be that the S251X and Y313X mutations resulted in a dominant negative effect. The extended spectrum of the WS4 phenotype is relevant to the brain expression of SOX10 during human embryonic and fetal development. Indeed, the expression of SOX10 in human embryo was not restricted to neural crest derived cells but also involved fetal brain cells most likely of glial origin. These data emphasize the important role of SOX10 in early development of both neural crest derived tissues, namely melanocytes, autonomic and enteric nervous systems, as well as in glial cells of the central nervous system.

Program Nr: 1959 from the 1999 ASHG Annual Meeting

Cat-like cry associated with a familial 5p terminal deletion distal to the cri-du-chat syndrome critical region. *L.C. Tuttle, X. Li, J.B. Ravnan, L. Bros, S. Owen, J. Kobori.* Genetics Department, Kaiser Permanente Northern California, San Jose, CA.

The existence of a cat-like cry critical region distal to the cri-du-chat critical region on chromosome 5p has previously been proposed. We report a family with an inherited 5p terminal deletion associated with a cat-like cry and variable degrees of intellectual impairment. N.C. was first seen as an inpatient at 3 days of age. She was noted to be SGA and hypotonic, with brachycephaly, full cheeks, microretrognathia, poor neurologic responsiveness and a high pitched cry. Cytogenetic studies revealed a small terminal deletion of 5p, 46,XX,del (5) (p15.2). Fluorescence in situ hybridization (FISH) using a DNA probe containing the cri-du-chat critical region at band 5p15.2 (D5S23, Oncor, Inc.) revealed a normal pattern, indicating that the deletion is distal to the probe locus. FISH analysis using a 5p telomeric probe (T5p5q, Cytocell Ltd.) confirmed a terminal deletion. Parental cytogenetic studies revealed the same deletion in the mother, M.O., a 34 year-old with a history of learning disability but no birth defects or medical problems. She has two other children, J.B. and S.C. J.B., an 8-year-old maternal half-brother to the proband, had been diagnosed with cerebral palsy. He has dysmorphic features including medial flare of eyebrows and broad nose, and significant developmental delay. S.C., a full sibling to the proband, is a non-dysmorphic 5-year-old girl with normal development but recently noted to have difficulty concentrating in school. Cytogenetic analysis revealed the presence of the same 5p deletion in both J.B. and S.C. The mother recalls that J.B. and S.C. also exhibited similar, high-pitched cries as infants. In conclusion, additional FISH studies are warranted on patients with cat-like cry and features not typical of classical cri-du-chat syndrome who have negative FISH studies for the cri-du-chat critical region. This report also illustrates the milder phenotypes in individuals with a more distal 5p terminal deletion than that which causes the classical cri-du-chat syndrome.

Program Nr: 1960 from the 1999 ASHG Annual Meeting

Dysostosis spondylocostal: a clinical study of 14 patients. *C.Y. Utagawa[1], C.A. Kim[1], S.M.M. Sugayama[1], D. Bertola[1], L.A.N. Oliveira[2], C.H. Gonzalez[1].* 1) Dept. of Pediatrics, Instituto da Crianca, University of Sao Paulo, Sao Paulo, Brazil; 2) Dept. of Radiology, Instituto da Crianca, University of Sao Paulo, Sao Paulo, Brazil.

Spondylocostal dysostosis is a skeletal disorder characterized by short trunk and short stature due to multiple vertebral and rib segmentation defects. There is an evident genetic heterogeneity with two patterns of inheritance: autosomal recessive and autosomal dominant. We report on clinical data from 14 patients (7 females and 7 males) belonging to 13 families. The age of the diagnosis ranged between 11 days and 6 years. Only 4/14 (28%) cases were diagnosed during the neonatal period. Half of the patients presented short stature at birth, while scoliosis and ribcage deformity were present in all cases. Recurrent pneumonias occurred in 6/14 (43%) and respiratory failure in 4/14 (28%). This latter event was the cause of death in one of these children during the neonatal period. Hernias (64%), neural tube defects (36%), congenital heart disease (14%) and urinary tract anomalies (14%) were the most frequent associated anomalies. Cerebral poligiria and delayed psychomotor development were found in only one child. The main skeletal malformations were hemivertebrae, fusion and hypoplasia of the vertebral bodies, agenesis, hypoplasia and fusion of the ribs. Dorsal column was affected in 92% of the patients, followed by lumbar (78%) and cervical region (43%). Progression of the scoliosis was present in 23% of the cases. Patients with extensive involvement of the column showed a less severe degree of scoliosis compared to the ones with mild vertebral anomalies. The children's parents were evaluated and none of them presented vertebral anomalies. Consanguinity was observed in two occasions and there was a recurrence (two sibs affected) in another one, suggesting a pattern of autosomal recessive inheritance in these families. Therefore, the majority of the cases (72%) were sporadic, in concordance to the literature.

Program Nr: 1961 from the 1999 ASHG Annual Meeting

A syndrome of multiple exostoses, hirsutism, mental retardation and seizures in a patient with a 8q24 submicroscopic interstitial deletion. *L. Van Maldergem[1], W. Wuyts[2], M. Foulon[3], C. Wetzburger[3], D. Roland[1], Y. Gillerot[1], W. Van Hul[2]*. 1) Medical Genetics, Inst Pathology & Genetics, Gerpinnes, Belgium; 2) Centrum Medische Genetica, Universitaire Instelling, Antwerpen, Belgium; 3) Department of Pediatrics, Centre Hospitalier Universitaire, Charleroi, Belgium.

Multiple exostoses is a genetically heterogeneous condition with currently two genes being identified : EXT1 in 8q24 and EXT2 in 11p11-12. The exostoses can occur isolated or as part of a syndrome. Multiple exostoses and mental retardation are part of the trichorhinophalangeal syndrome type II (TRP II) which is caused by a deletion of the region encompassing the EXT1 gene. We describe a single patient born to unrelated Tunesian parents with multiple exostoses, mild mental retardation, hirsutism, a porencephalic cyst and seizures. Interestingly, this patient doesn't have the classical symptoms of TRP II since no cone shaped epiphyses are present on hand radiograph and no pear shaped nose or sparse hair are observed. By contrast, he has a striking hypertrichosis of the face with bushy eyebrows. FISH was performed with probes for the EXT1 gene (8q24) and EXT2 gene (11p11-p12). This analysis revealed the presence of a deletion of the EXT1 probe on chromosome 8q24. Further PCR analysis with polymorphic markers surrounding the EXT1 gene showed a deletion in the paternal chromosome flanked by the markers D8S547 (122,6cM) and D8S1728 (127,6cM), including the entire EXT1 gene. This case only resembles a single case report described by Wiedemann et al. (Am J Med Genet 46:403-409; 1993) with hirsutism and exostoses in a 19 year-old male. However, the topography of hypertrichosis from this patient (the trunk but not the face) and also the facial dysmorphia appear different. We suggest that the unique association in our patient of facial hirsutism, dysmorphia, mental retardation, seizures and multiple exostoses represent a new contiguous gene syndrome that partially overlaps with TRP II.

Program Nr: 1962 from the 1999 ASHG Annual Meeting

A clinical, cytogenetic and molecular study of three Brazilian patients with supernumerary inv dup(15) marker chromosomes. *M.C. Varela[1], C. Fridman[1], T.E. Matsumoto[1], C.A. Kim[2], F. Kok[3], A. Diament[3], C.P. Koiffmann[1]*. 1) Dept Biology, University of Sao Paulo; 2) Child Unit, Child Institute, Faculty of Medicine; 3) Child Neurology Service, University of Sao Paulo, School of Medicine, Sao Paulo, Brazil.

The presence of supernumerary marker chromosomes (SMC) is not rare and the inv dup(15) is the most common (~0,02% of liveborn). Patients with inv dup(15) containing the Prader-Willi (PWS)/Angelman syndrome (AS) critical region usually have hypotonia, mental retardation, seizures, growth retardation and autism. Here we present two girls (9 and $8^{5/12}$y) and one boy (2y) with inv dup(15). Banding GTG studies disclosed the presence of a bissatellited SMC that had the size of a G-group chromosome. Parental karyotypes were normal. FISH using the probes D15Z1 (a-satellite probe) and single-copy sequences that map to 15q11-q13 (GABRB3 and SNRPN) disclosed two signs on the SMC, and normal chromosome 15 homologues. The analysis of SNRPN methylation was normal, excluding the AS or PWS diagnosis. The girls showed an increased intensity of the maternal band indicating the maternal origin of the extra chromosome that was confirmed through the microssatellite analysis (D15S542, D15S11, D15S113, GABRB3); these markers were not informative in the boy and additional loci will be tested. All three probands showed delayed motor development, mental retardation, seizures, lack of speech and ataxic gait. The girls presented growth retardation (height £3rd; weight £10th) and outsburts of laughter. Hypotonia, microcephaly, brachycephaly, macrostomia and hyperactivity was presented in 1 girl. The boy showed normal growth patterns (height and weight - 75thcentile), no hyperativity and no outsburts of laughter. All of the probands have 4 copies of the PWS/ASCR; the girls also have a physical excess of maternal versus paternal alleles of these region as well as an increased maternal dosage of this imprinted region. The phenotypic variability observed among patients with inv dup(15) is influenced by the origin of the marker, the extend of the euchromatic segment and by its isodysomic or heterodysomic nature. Supported by FAPESP.

Program Nr: 1963 from the 1999 ASHG Annual Meeting

OSTEOPATHIA STRIATA WITH CRANIAL STENOSIS : PHENOTYPIC SPECTRUM AND SUGGESTION OF X-LINKED INHERITANCE. *G. VIOT[1], D. LACOMBE[2], A. DAVID[3], D. SANLAVILLE[1], M. MATHIEU[4], A. de BROCA[4], A. MUNNICH[1], S. LYONNET[1], M. LE MERRER[1], V. CORMIER-DAIRE[1]*. 1) Department of Genetics, Hopital Necker AP-HP, PARIS, FRANCE; 2) Department of genetics, Hopital Pellegrin, BORDEAUX, FRANCE; 3) Department of genetics, Hopital Mere-enfant, NANTES, FRANCE; 4) Department of genetics, Hopital NORD, AMIENS, FRANCE.

Osteopathia striata with cranial sclerosis (OS-CS) is a rare autosomal dominant multiple congenital anomaly syndrome including dysmorphic features with frontal bossing, hypertelorism, cleft palate, macrocephaly, hearing loss and mild mental retardation. The diagnosis is based on radiological findings namely longitudinal striations of long bones metaphyses and cranial sclerosis. Here we report on 10 OS-CS patients, including 2 sporadic and 4 familial cases. In each of the patients macrocephaly and typical craniofacial appearance were present at birth, and radiographic survey confirmed the diagnosis. In addition to the classical findings, marked phenotypic variability was observed. In particular, 3 cases presented a cardiac anomaly (VSD, PDA or cardiomyopathy). Cerebral ventricular dilatation with normal pressure was noted in 3 cases, and hydrocephaly with coronal craniosynostosis required surgical treatment in one patient. Corpus callosum hypoplasia, Pierre Robin sequence, hearing deficiency, irregular teeth and scoliosis were noted in 2/10 patients. One patient presented with Hirschsprung disease, which has never reported in the syndrome thus far. Psychomotor development was retarded in 7/10 cases. Finally, chromosome analysis could be performed in 6/10 cases and was normal. Our series suggests both inter- and intra-familial variable expression of OS-CS. Although severe motor retardation could be observed during the first years of life, long term follow-up showed a significant improvement. Interestingly, we found a marked skewed sex ratio with high excess of affected females (9F/1H), suggesting that OS-CS might behave as a dominant X-linked trait with male lethality, especially as the only male patient was severely affected with cardiomyopathy, corpus callosum hypoplasia, Hirschsprung disease and mental retardation.

Program Nr: 1964 from the 1999 ASHG Annual Meeting

Kaposiform hemangioendothelioma and cerebral cortical dysplasia in a newborn with Prader-Willi syndrome caused by maternal UPD 15. *J. Wagstaff, H. Kozakewich, J.B. Mulliken, B.-L. Wu, V. Lip, A. Duplessis.* Children's Hospital, Boston, MA.

Maternal UPD 15 causes approximately 30% of cases of Prader-Willi syndrome, as a result of lack of an active paternal copy of one or more imprinted genes from 15q11-q13. A female infant was born to a 41-year-old mother after a pregnancy complicated by decreased fetal movements. At birth, the infant had a massively enlarged left thigh, marked hypotonia, and profound thrombocytopenia. Biopsy of the thigh mass showed kaposiform hemangioendothelioma; the tumor was treated with interferon alpha-2b for 10 months, with resolution of the tumor and thrombocytopenia. Karyotype was 46,XX on amniotic fluid, peripheral blood, and the hemangioendothelioma. Brain MRI showed focal areas of thickened cortex within the left precentral gyrus, right frontal lobe, and posterior right parietal lobe. Methylation analysis with PW71B showed a maternal-only pattern, and polymorphism analysis showed maternal UPD 15. The findings of a rare vascular tumor and cerebral cortical dysplasia in a child with Prader-Willi due to maternal UPD 15 have not been reported previously. Possible explanations for these associated findings include homozygosity for recessive alleles on chromosome 15, trisomy 15 mosaicism, or a cryptic chromosomal anomaly (e.g., translocation) associated with loss of the paternal chromosome 15 during early development.

Program Nr: 1965 from the 1999 ASHG Annual Meeting

The evolving clinical phenotype of Rothmund-Thomson Syndrome. *L.L. Wang[1], M.L. Levy[1], R.A. Lewis[1], M.M. Chintagumpala[1], L.R. Carlson[2], C.M. Cunniff[3], S.C. Elliott[2], M. Frydman[4], S.E. Iden[3], D.M. Koeller[5], S.E. Plon[1], D. Lev[6], M. Rogers[7], S.P. Yang[8]*. 1) Baylor College of Medicine, Houston, TX; 2) Blank Children's Hospital, Des Moines, IA; 3) U. of Arizona HSC, Tucson, AZ; 4) Chaim Sheba Medical Center, Tel Hashomer, Israel; 5) U. of Colorado HSC, Denver, CO; 6) Wolfson Medical Center, Holon, Israel; 7) New Children's Hospital, Sydney, Australia; 8) UC Davis Medical Center, Davis, CA.

Rothmund-Thomson Syndrome (RTS) is a rare autosomal recessive genodermatosis characterized by a persistent rash that starts in infancy, small stature, skeletal abnormalities, sparse hair, bilateral juvenile cataracts, and predisposition to developing skin and bone cancers. RTS was first described by Rothmund (1868) in a large consanguinous Austrian kindred. His patients had rapidly progressive bilateral juvenile cataracts and a peculiar rash. Since then approximately 250 cases of RTS have been described in the English literature, 17 with osteosarcoma (OS) (~7%). As part of a study to determine the molecular basis of RTS we have identified a contemporary cohort of 23 patients with RTS (ages 2-35 years), ascertained by referral from dermatology, ophthalmology, genetics, and oncology. 8 patients have had OS, 3 have radial dysplasias, but none by family and/or physician report has developed cataracts or skin cancer. Concordance of OS in siblings from 2 families was observed. 2 cases of OS were multifocal. Of the 15 patients without OS, 12 are less than 15 years-old and thus remain at risk for developing this tumor. Ascertainment bias may influence the high rate of OS. However, 3 of 5 patients originally diagnosed with RTS as young children at Baylor have subsequently developed OS. The clinical profile of our patients, with a high rate of OS and low rate of cataracts, suggests a different phenotype from Rothmund's patients, and may reflect either allelic heterogeneity at the RECQL4 locus or genetic heterogeneity. These findings also suggest that consideration be given to screening for OS during adolescence as part of the clinical management of RTS.

Program Nr: 1966 from the 1999 ASHG Annual Meeting

Microcephalic osteodysplastic primordial dwarfism type I in the Amish. *J.A. Westman[1], E.H. Stover[1], C. Singley[2].*
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Microcephalic osteodysplastic primordial dwarfism type I (OMIM #210710) is a rare syndrome characterized mainly by low birth-weight dwarfism, extreme microcephaly, a prominent nose and eyes, sparse scalp hair, a short neck, short limbs with dislocation of the elbows and hips, relatively broad hands and feet and dry hyperkeratotic skin. Less than 15 cases have been previously described in the world literature. We report four affected infants in two Ohio Amish families, providing further evidence for the autosomal recessive nature of the syndrome. The genetic and/or biochemical cause of the syndrome has not been established. Electron microscopy of skin fibroblasts from the most recently born child did not show any cytoplasmic inclusions which might indicate the presence of a storage disorder. All four parents of the two Amish families are related through multiple family lines. An eight to twelve generation pedigree analysis extending back to the initial Amish-American immigration limits the likely founder mutation to one or more of 8 families and assists in interpretation of future linkage analysis. Additional Amish families with affected children are being identified.

Program Nr: 1967 from the 1999 ASHG Annual Meeting

Genetic assessment of mentally retarded dysmorphic adults with primary psychiatric diagnoses. *M.S. Williams[1], C.L. Wood[2], A.C. Wendorff[3]*. 1) Dept Pediatrics, Gundersen Lutheran Medical Ctr, La Crosse, WI; 2) Dept Psychiatry, Gundersen Lutheran Medcical Ctr, La Crosse, WI; 3) Gundersen Lutheran Medical Foundation, La Crosse, WI.

We report the results of evaluations of 23 mentally retarded adults referred for suspected genetic disease. Patients ranged in age from 19 to 48 years. There were 12 males and 11 females. All had at least one primary psychiatric diagnosis. Impulse control disorder, NOS was the most common psychiatric diagnosis. Other common diagnoses included bipolar disorder, schizophrenia and dysthymia. Only seven patients had had a previous evaluation by a geneticist. Four had a specific genetic diagnosis, of which two were confirmed, one ruled out and one in which an additional (causative) diagnosis was added. Specific genetic diagnoses were made in five additional patients and suspected diagnoses in three others. Private or familial syndromes were suspected in five patients, although evaluation of other putatively affected individuals was not accomplished.

We will present clinical vignettes including pictures and diagnostic modalities employed to arrive at specific diagnoses. The potential value of genetics evaluation in this patient population will be discussed.

Program Nr: 1968 from the 1999 ASHG Annual Meeting

Three patients with Autistic Disorder and isodicentric chromosome 15: Implications for genetic counseling. *C.M. Wolpert[1], M.M. Menold[1], M.P. Bass[1], M.C. Qumsiyeh[2], S.L. Donnelly[1], S.A. Ravan[3], R.K. Abramson[3], H.H. Wright[3], L.O. Maddox[1], J.M. Vance[1], J.R. Gilbert[1], M.L. Cuccaro[3], M.A. Pericak-Vance[1]*. 1) Dept Medicine, Duke Univ Medical Ctr, Durham, NC; 2) Dept Pathology, Duke Univ Medical Ctr, Durham, NC; 3) W.S. Hall Psychiatric Institute, Univ of South Carolina, Columbia, SC.

Isodicentric chromosome 15 anomalies encountered in clinical practice can be problematic when providing phenotypic information due to wide variation in clinical presentations of these patients. This includes recent research findings implicating the proximal 15q region in the genetics of Autistic Disorder (AD). This evidence includes 1) proximal 15q anomalies are the most frequently reported cytogenetic anomalies in AD patients; 2) several genetic-mediated, developmental disorders that can include autistic-like behaviors map to this region, including Prader Willi Syndrome and Angelman Syndrome; 3) Genomic-wide linkage screens identified 15q11-13 as a candidate region housing potential AD susceptibility genes; 4) linkage disequilibrium with AD has been reported in this region. We hypothesize those individuals with AD and an isodicentric chromosome 15, encompassing the 15q11.2 region, will share physical and developmental findings. Three unrelated patients were identified through family ascertainment efforts for a gene identification study for AD. All three patients met DSM-IV/ICD-10 diagnostic criteria for AD, confirmed by the Autism Diagnostic Interview, and had neurological examinations. Cytogenetic analysis revealed the following karyotypes: 47XX, idic (15q11.2), 47XX, idic (15q11.2), and 47XY, idic (15q11.2). Comparison of these 3 patients with published case reports (N=17) reveals a clustering of physical and developmental features. Specifically, all patients exhibited hypotonia, seizures, and delayed gross motor skills. All three of our patients and a majority of patients in the literature exhibited speech delay, lack of social reciprocity, abnormal eye contact, and stereotyped behaviors. This data may provide more detailed information for families of individuals with both AD and isodicentric 15q anomalies.

Program Nr: 1969 from the 1999 ASHG Annual Meeting

Hirschsprung-like aganglionosis of the colon in patients with nail patella syndrome: Extension of the NPS phenotype to a fourth organ system. *M.J. Wright, I. McIntosh.* Greenberg Center for Skeletal Dysplasias & Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD.

Nail patella syndrome (NPS) is a pleiotropic condition characterized by dysplasia of the nails, hypoplasia of the patellae, elbow dysplasia and progressive kidney disease. In addition, open-angle glaucoma has recently been shown to be part of the phenotype. The syndrome is inherited in an autosomal dominant manner and has been shown to result from mutations in the LIM-homeodomain encoding LMX1B gene. A number of individuals affected with NPS describe symptoms of severe constipation, often from birth, and often requiring medication. A proportion of these had been diagnosed with megacolon. Together these findings are suggestive of short-segment Hirschsprung disease. One 16 year old male with a history of constipation from birth, with failure to pass meconium until 2 weeks of age, presented with recurring fecal impaction. Barium enema demonstrated enlargement of the rectum and left colon consistent with the diagnosis of secondary megacolon. Transmural rectal biopsy performed at analrectal strip myomectomy showed aganglionosis and fibrosis of the muscularis propria confirming the diagnosis of aganglionic megacolon. Anecdotal evidence would suggest that aganglionic megacolon may be as common and as clinically significant among patients with NPS as kidney disease or glaucoma. Of interest, it has recently been shown that the *C. elegans* ortholog of LMX1B, *lim-6*, regulates the outgrowth and differentiation of neurons controlling rhythmic enteric muscle contractions. *lim-6* mutant animals exhibit a bloated gut and defective defecation behavior. In conclusion, we suggest that assessment of individuals with NPS should include direct questioning regarding symptoms of constipation to allow definitive diagnosis of this treatable complication.

Program Nr: 1970 from the 1999 ASHG Annual Meeting

Benign familial infantile spasms syndrome (BFISS) with autosomal dominant inheritance. *K. Yasuda[1], K. Fujii[1], J-i. Takanashi[1], T. Fukuda[2], K. Obata[2], H. Yamagata[2], Y. Kohno[1], I. Kondo[2].* 1) Dept. of Pediatrics, Shcool of Med., Chiba Univ., Chiba 260-8670, Japan; 2) Dept. Hygiene, Ehime Univ. School of Med., Ehime 791-0295, Japan.

West syndrome (WS) is an age-related epileptic syndrome of infancy and the classical triad of the WS are 1) infantile spasms (IS), 2) electroencephalogram (EEG) pattern of hypsarrythmia, and 3) mental retardation. Prognosis is generally considered to be poor and seizures often persist. Some patients with the WS, however, recover completely and have a normal intellectual outcome. The WS is etiologically heterogeneous. Although most patients with WS are sporadic, a possible hereditary predisposition has been considered. Recently, the disease locus of an X-linked IS (WS) has been mapped to Xp21.3-p22.1. We report a family of 5 affected individuals (two males and three females) with IS in two generations. All affected members first had epileptic attacks between the ages of 3 and 4 months. Hypsarrhysmia was detected in EEG findings, but affected members recovered completely after adrenocorticotropichormone (ACTH) therapy and have a normal neurodevelopmental status. Male twins in the family had the same clinical characteristics. All patients had no cutaneous and focal neurological abnormality. Laboratory findings were normal and the magnetic resonance imaging (MRI) scan showed no underlying neurologic disorder. Clinical prognosis and mode of inheritance in our family differ from those of the X-linked IS. Our family suggests a new infantile spasms syndrome with an autosomal dominant inheritance, a benign familial infantile spasms syndrome (BFISS).

Program Nr: 1971 from the 1999 ASHG Annual Meeting

***De novo* t(3;4)(q21;q31) translocation in an infant with spinal muscular atrophy type I (Werdnig-Hoffmann disease) and no deletion of SMN1.** *A. Zankl, M.-Cl. Addor, D.F. Schorderet.* Division of Medical Genetics, CHUV, Lausanne, Switzerland.

Spinal muscular atrophy (SMA) is the second most common lethal, autosomal recessive disease in Caucasians after cystic fibrosis. The disorder is characterized by degeneration of the anterior horn cells of the spinal cord, leading to symmetrical weakness and wasting of muscles. Three types can be distinguished: type I (Werdnig-Hoffmann disease) is a severe form with onset of symptoms in the first 6 months of life and death due to respiratory failure usually occuring within 2 years; type II is an intermediate form; type III (Kugelberg-Welander disease) takes a more benign course with onset of symptoms in late childhood and slow progression. All three types have been mapped to 5q11.2-13.3 and seem to be caused by alterations in the SMN1 gene. Up to 98.6% of patients with SMA show homozygous deletion of exon 7 (Lefebvre et al., 1995). In most of the remaining cases other mutations of SMN1 have been identified. However, Wirth and coworkers (1999) found no deletion or mutation of SMN1 in 14 of 525 SMA patients, suggesting the existence of SMN-unlinked SMA.

We report on an infant who was investigated for hypotonia and muscle weakness and died at 10 months of age from respiratory failure. He was diagnosed clinically as having Werdnig-Hoffmann disease fulfilling the inclusion and none of the exclusion criterias for SMA set forth by the International SMA Consortium in 1992. No deletion of exons 7 or 8 of SMN1 could be detected in this patient. However, his karyotype revealed an apparently balanced t(3;4)(q21;q31) translocation. Karyotypes of the parents were normal, indicating a *de novo* event. To our knowledge, this is the first report of a patient with SMN-undeleted Werdnig-Hoffmann disease and a chromosomal aberration. We conclude that genes in 3q21 or 4q31 might play a role in the development of spinal muscular atrophy. Molecular characterization of the chromsome breakpoints observed in our patient will allow a better understanding of the gene(s) involved in SMA.

Program Nr: 1972 from the 1999 ASHG Annual Meeting

A syndrome with autosomal recessive polycystic kidneys with skeletal and facial anomalies is not linked to the ARPKD gene locus on chromosome 6p. *K. Zerres[1], N. Kohlschmidt[2], G. Muecher[1], C. Hallermann[2], P. Shahidi[2], B. Welleck[3], U. Theile[3], R. Schumacher[4], F. Bahlmann[5], S. Rudnik[1], H. Muentefering[2]*. 1) Institute for Human Genetics, Technical University of Aachen, Germany; 2) Department of Pediatric Pathology, University of Mainz, Germany; 3) Genetic Counseling Unit, Mainz, Germany; 4) Department of Pediatric Radiology,University of Mainz, Germany; 5) Department of Gynecology, University of Mainz, Germany.

We report on two siblings, one male born at 37 weeks and one male fetus of 24 weeks of gestation, both with a syndrome similar to that seen in three sets of siblings by Gillessen-Kaesbach et al. (Am J Med Genet 45:511-518, 1993). Both proposity had polycystic kidneys and hepatic fibrosis indistinguishable from autosomal recessive polycystic kidney disease (ARPKD), plus skeletal malformations and facial anomalies. Skeletal abnormalities included butterfly vertebrae, square shape of the pelvis and brachymelia. The facial anomalies included hypertelorism, epicanthic folds, and anteverted nares. Additional external findings were low set ears and short neck. Histopathological examination of the kidneys of the neonate showed radial orientation and cystic dilatation of the cortical and medullar tubules. The liver showed "congenital hepatic fibrosis". The hepatic findings in the fetus were less severe. Renal abnormalities were limited to focal tubular cystic changes. Linkage analysis with polymorphic markers of the region 6p21.1-p12, flanking the gene locus of ARPKD, showed different haplotypes in both siblings, thus excluding the ARPKD gene locus in this family and indicating genetic heterogeneity.

Program Nr: 1973 from the 1999 ASHG Annual Meeting

Telomeric Translocations with Abnormal Phenotypes. *C.B. Lozzio, E. Bamberger, T. Ryan, E. Holland, C. Worthington, I. Anderson.* Develop & Genetic Ctr, Med Ctr, Univ Tennessee, Knoxville, TN.

Translocations involving the telomeric regions 7q and 11p were observed in cases with apparently balanced karyotypes and abnormal phenotypes. Two cases had de novo rearrangements including the tel 7q and one was an inherited translocation with normal phenotype in the mother and abnormal phenotype in two fetuses who had inherited the same apparently balanced translocation involving the telomeric region 11p15.5. The two cases with the de novo rearrangements of the tel 7q included , one with a complex rearrangement and one with a reciprocal translocation. The complex rearrangement had four breaks, at bands 7q32, 7q35, 2p12 and 2q31. Cytogenetic studies at band level 800 and FISH studies with wcp 2 and wcp 7 showed the translocation of the region 2p12®2ptr to band 7q32 in the derivative chromosome 7 and an insertion of the region 7q32-q35 to 2q31 in the derivative chromosome 2. Only the FISH studies with the telomeric probe 7q showed the translocation of tel 7q to band 2p12 in the derivative chromosome 2. This rearrangement was observed in a 15 month old girl with infantile spasm, severe developmental delay, marked hypotonia, microbrachycephaly and mild dysmorphic features. Another de novo translocation t(7;8)(q36.3;q22.3) was found in a child with cleft lip and palate, hypodontia, microcephaly, malrotation of the intestines and complex partial seizures. The translocation of tel7q to the derivative chromosome 8 was only found with the tel 7q probe and not with wcp 7 and wcp 8. An inherited translocation t(4;11)(q23;p15.3) was found in an amniocentesis of a fetus with omphalocele. The normal mother was a balanced carrier of the translocation and she had a previous fetus who died at 25 weeks with omphalocele. FISH studies with the tel 11p probe showed that the 11p15.5 region containing the genes for Beckwith Wiedemann Syndrome (BWS) was translocated to the derivative chromosome 4. The possibility that maternal imprinting of the BWS could explain the two fetuses with omphalocele will be considered. The implications of position effect and/or molecular deletions and/or duplications causing abnormal phenotypes will be discussed.

Program Nr: 1974 from the 1999 ASHG Annual Meeting

An abnormal fetus with trisomy 20 and trisomy 9 mosaicism. *J.K. Lundberg, M.G. Bialer, A. Yenamandra, L. Trinchitella, E.. Schneider, R. Perrone, X. Zhou, E. Kahn, P. Koduru.* Dept. of Pediatrics, Laboratory and OB/GYN, North Shore Univ. Hospital/NYU School of Medicine, Manhasset, NY.

A 39 year old Saudi Arabian/Asian Indian woman was seen for genetic counseling for advanced maternal age and consanguinity (1st and 2nd cousins). Family history was significant for a son with pulmonic stenosis. Screening revealed that she is an a-thalassemia carrier. At 15 3/7 wk a comprehensive ultrasound revealed bilateral cerebral ventriculomegaly, dilated cisterna magna, bilateral cystic lesions in the anterolateral neck, and pleural effusion. On amniocentesis, AF-AFP was normal, but chromosome analysis of 20 metaphases from in-situ cultures of the amniotic fluid showed 47,XY,+20. Because this is usually a mosaic finding, we counted additional cells. Of 56 total cells counted, 50 were 47,XY,+20 and 6 were 47,XY,+9. No normal cells or double trisomy cells were seen. Pregnancy was terminated by Prostin induction at 18 wk. Post-mortem examination revealed cystic hygroma, low-set ears, micrognathia, truncus arteriosus, VSD, left diaphragmatic hernia, and hypoplastic left lung. Microscopic examination of the skin revealed lymphangiectasia. Chromosome analysis of 20 metaphases each of cells cultured from placenta, umbilical cord, heart, blood, skin, lung, liver, right and left kidney (total of 160 metaphases) all showed a combination of trisomy 9 and trisomy 20 cells with no normal or double trisomy cells, except lung had only trisomy 20 cells. The clinical findings are consistent with trisomy 9. Trisomy 20 mosaicism is usually benign and not confirmed in blood. Trisomy 20 cells in blood may have phenotypic consequences, but this has not been proven. It is not clear how these chromosome anomalies arose. If there had been 2 separate non-disjunction events, there should be a normal cell line. If the pregnancy had started with a single trisomy and a second non-disjunction occurred, with subsequent loss of one of the original trisomic chromosomes, then a cell line with double trisomy should exist. If both trisomies were the result of an autosomal recessive condition predisposing to karyotypic instability, then additional abnormal cell lines would be expected.

Program Nr: 1975 from the 1999 ASHG Annual Meeting

Varations of the heterochromatic segment of non-fluorescent Y-chromosome [Yq^{nf}] as revealed by FISH-technique. *M.J. Macera[1], R.S. Verma[1], N. Merhi[2], H. Wehbeh[2].* 1) Wyckoff Heights Medical Center, Brooklyn, NY. Institute of Molecular Biology and Genetics at InterScience, SUNY Health Science Center at Brooklyn, NY; 2) Luthern Medical Center, Brooklyn, NY.

The major variation of the long arm of human chromosome Y is accounted for by the heterochromatic segment that fluoresces brightly by quinacrine. This variation was established immediately after the advent of the QFQ-banding technique. Later, the variation of the non-fluorescent segment was also noted [Verma et al. J Med Genet 15:277-281, 1978]. Earlier, we reported a prenatal case that has a non-fluorescent Y by QFQ-staining, however, using the FISH technique with the Y-cocktail probe [DYZ1/DYZ3](Oncor), which is specific for satelite III and alpha satellite heterochromatin, a small amount of satellite III DNA was detected in both fetal Y and the father showing that the Yq^{nf} chromosome had heterochromatin [Silverman et al Genet Med 1:70; 1999]. Recently, we were referred another case for genetic amniocentesis that had a Yq^{nf}, by QFQ, in amniocytes. The biological father also posseses the Yq^{nf} chromosome by QFQ staining. However, when FISH was employed with the Y-cocktail probe, no signals were observed at the DZ1 location, suggesting another variation identified by the FISH technique. The second Yq^{nf} is obviously different from the first one. The satellite III sequences are completely absent, or more likely, the number of satellite III sequences present is below the detection level of the probe. The mothers in both cases experienced one fetal loss. These two Yq^{nf}'s were discovered from approximately 1000 cases evaluated prenatally. Their clinical significance is unknown but may serve as phylogenetic markers for those who are in search of family lineage using the Y-chromosome for haplotyping.

Program Nr: 1976 from the 1999 ASHG Annual Meeting

Characteristics of two cases of DUP(15)(Q11.2-Q12). *R. Mao[1], S.M. Jalal[1], K. Snow[1], V.V. Michels[1], S.M. Szabo[2], D. Babovic-Vuksanovic[1]*. 1) Mayo Clinic, Rochester, MN; 2) LaSalle Clinic, Oshkosh, WI.

We describe the clinical, cytogenetic, and molecular findings of interstitial duplication of 15q11.2-q12 in two unrelated patients. By high resolution chromosome analysis patient one was normal and duplication was suspected in patient two. The duplication was established in both patients by metaphase FISH using two critical region (SNRP-N and D15S10) probes and their control probes. The parents of patient one were normal by FISH analysis but the parents of patient two were unavailable. Southern blot analysis based on parent of origin specific DNA methylation at D15S63 (PW71) locus detected a difference of intensity of methylated and unmethylated fragments. The molecular pattern suggested a maternal origin of the duplication in patient one and a paternal origin in patient two. Patient one (2 years old) had developmental and speech delay with pervasive developmental disorder or mild autism, strabismus, and normal growth parameters with seizures. Patient two (16 years old) had global developmental delay, verbal I.Q. of 94, depression, obesity, food-seeking behavior, and significant behavioral problems that included self-injurious tendencies. Neither patient had significant dysmorphic features or abnormalities of internal organs. It has been suggested that patients with duplication in the PWS/AS critical region of maternal origin tend to have milder features of Angelman syndrome and those of paternal origin may have milder features of Prader-Willi syndrome, although this has not always been true. Our two cases suggest that some patients with 15q11.2q12 duplication may have significant anomalies, and there appears to be phenotypic differences between maternal and paternal transmission of the anomaly. Role of imprinted loci and gene dosage in these duplications remains speculative.

Program Nr: 1977 from the 1999 ASHG Annual Meeting

Two unrelated cases of (2q;12q) balanced translocation sharing abnormal features and learning disability. *K. Marks[1], L. Telvi[2], A.M. Ion[2], J.C. Carel[3], J.L. Chaussain[3], M.A. Patton[1].* 1) Medical Genetics, St George's Hospital, London, United Kingdom; 2) Genetics Department, St Vincent de Paul Hospital, Paris, France; 3) Paediatric Endocrinology, St Vincent de Paul Hospital, Paris, France.

We report two unrelared cases presenting with a balanced translocation involving the chromosomal band 12q15: 46,XY,t(2;12)(q22;q15) and 46,XX,t(2;12)(q32;q15),inv(6)(p21.1;p23) respectively. Common features in both patients included feeding problems in early childhood, short stature, moderate learning difficulties, cubitus valgus, squint, short and prominent filtrum, full lips, high arched palate and micrognatia. Chromosomal rearrangements occured de novo (normal parents' karyotype), and detailed cytogenetic analysis, including high resolution chromosomes and FISH techniques showed that no other chromosome was involved in either of the balanced translocations. Since a common break-point was found on the chromosome 12 but not on the chromosome 2, common features in the two patients may be due to an abnormal gene functioning at 12q15. Both patients had been referred for Noonan syndrome, but this could not be clinically confirmed due to the lack of full diagnostic criteria, such as heart abnormalities. However, suggestive features for this disorder were present in both cases. This condition has been previously mapped at 12q24.1, but a number of familial cases do not appear to be linked to this locus, suggesting a degree of heterogeneity or a more complex aetiology. The cytogenetic and molecular DNA analysis of the present reported cases, suggest the possibility of a second locus involved in Noonan syndrome.

Program Nr: 1978 from the 1999 ASHG Annual Meeting

Partial trisomy 9 due to 3:1 segregation of a maternal 9;14 translocation. *S.L. Marles[1,3], T. Sudha[3], J. De Nanassy[4], D.E. Wickstrom[2], D. Konkin[2], A.J. Dawson[1,2,3].* 1) Section of Genetics and Metabolism, HSC, Winnipeg, MB, Canada; 2) Cytogenetics Laboratory, HSC, Winnipeg, Manitoba, Canada; 3) Dept. Human Genetics, University of Manitoba, Winnipeg, Manitoba, Canada; 4) Dept. Pathology, HSC, Winnipeg, Manitoba, Canada.

Tertiary trisomy is uncommon and generally occurs when the derivative chromosome is of small genetic content. Partial trisomy 9 has been reported previoulsy with the most common clinical abnormalities including microcephaly, skeletal anomalies, hand abnormalities, malformed ears, a "bulbous" nose, and nail hypoplasia. We report a 29 yr old G3P1SA1 woman who was referred for amniocentesis because of a positive triple screen test in which the risk for trisomy 18 was 1/30. Cytogenetic analysis showed an unbalanced female karyotype in the fetus with an apparent terminal deletion of the long arm of chromosome 9: 47,XX,+del(9)(q22). The pregnancy was terminated at 22+ weeks gestation. Autopsy revealed facial, skeletal, and brain anomalies consistent with previous reports of partial trisomy 9. These anomalies included low set ears, micrognathia, a broad and bulbous nose with a prominent nasal bridge, aplasia of the distal phalynx of the right second toe, absence of the twelfth rib and under mineralization of the cervical vertebrae. Brain anomalies included large anterior and posterior fontanelles and wide sutures. There was no significant family history. Cytogenetic analysis of the mother showed a female karyotype with a balanced translocation between the long arms of chromosomes 9 and 14: 46,XX,t(9;14)(q22;q32). The chromosome anomaly in the fetus was the result of a 3:1 segregation of the maternal 9;14 translocation. The fetal karyotype was amended to: 47,XX,+der(9)t(9;14)(q22;q32)mat. The fetus did not show any clinical features which might be associated with duplication of the distal segment of 14q, suggesting that distal 14q does not contribute significantly to the phenotype. The charateristics associated with the meiotic configuration of the 9;14 translocation suggest that more than one type of malsegregation may be possible. This implies an atypically higher recurrence risk for a liveborn aneuploid child.

Program Nr: 1979 from the 1999 ASHG Annual Meeting

Molecular cytogenetic characterization of an unbalanced t(4p;21q) in a patient misdiagnosed as Full Monosomy 21. *L. Martelli[1,2], E.S. Ramos[2], C.G.C Lemos[1], V.M. Motta[1], S.A. Santos[2], T.A. Bernardes[1], A.C.C. Nassr[1], J.R. Coelho[1], J.E. Chufalo[1], S. Almeida[1], J.G. Franco Jr.[1].* 1) Division of Genetics,Sinha Junqueira Maternity Foundation,Ribeirao Preto,SP,Brazil; 2) Dept.Genetics, School of Medicine of Ribeirao Preto - University of Sao Paulo, Brazil.

Monosomy 21 is a rare aneuploid condition, generally considered to be incompatible with life. According to the literature, most of the reported cases may represent structural rearrangements resulting in only partial monosomy 21. The proband is the first child of non-consanguineous parents and was delivered at 31 weeks of gestation with a birth weight of 495g. Physical examination showed a peculiar facies, hypertelorism, high forehead with furrowing in central forehead, posterior midline scalp defects, cleft lip and palate, micrognathia, cardiac anomaly, simian crease, clubfeet and dorsiflexed hallus. He survived for 36 hours. Conventional cytogenetic analysis of peripheral blood lymphocytes revealed a 45,XY,-21 karyotype in 150 metaphases. Re-evaluation combining high resolution GTG banding and FISH analysis showed an unbalanced translocation characterized by 45,XY,-21,der(4)t(4;21)(p15.2;q21.1). The derivative chromosome presented the Down syndrome critical region (21q22.13-q22.2) and deletion of the region 4p16.3, justifying the clinical manifestations of the Wolf-Hirschhorn syndrome.We concluded that the patient presented a partial monosomy on both 4p and 21. The parental karyotypes including FISH studies were normal. Our findings confirm the need for complementary molecular cytogenetic investigations in cases of apparent monosomy 21.

Program Nr: 1980 from the 1999 ASHG Annual Meeting

Limitations of FISH analysis for prenatal diagnosis of chromosomal aneuploidies. *M.M. McCorquodale[1], J.K.B. Matheson[2], F.E. Tahmaz[3], T. Nguyen[2], M. Freidine[3].* 1) Michael Reese Hosp & Med Ctr, Chicago, IL; 2) Dept Ob/Gyn, Univ of Illinois, Chicago, IL; 3) Dept Peds, Univ of Illinois, Chicago, IL.

Following the FDA approval of the use of FISH probes, our laboratory has received a number of requests for prenatal diagnosis using the FISH technique without concurrent cytogenetic analysis. We recently experienced a case of a 29 year-old female referred at 26 weeks of gestation due to the detection of multiple congenital abnormalities by ultrasound. FISH analysis revealed no chromosomal aneuploidy for the sex chromosomes or for chromosomes 13, 18, and 21. Cytogenetic analysis, however, revealed the presence of an isochromosome 18q. The pregnancy was terminated and a detailed autopsy was performed. This case demonstrates that isochromosomes, cannot be detected by FISH using alpha satellite probes. FISH analysis should always be accompanied by concurrent karyotype analysis.

Program Nr: 1981 from the 1999 ASHG Annual Meeting

A candidate region for Coffin Siris - Syndrome at 7q32-34. *E.M. McGhee[1,2], C. Klump[2], S. Bitts[2], P.D. Cotter[1,2], E.J. Lammer[1]*. 1) Pediatrics - Medical Genetics, Univ California, San Francisco, San Francisco, CA; 2) Medical Genetics - Children Hospital, Oakland, CA.

Coffin - Siris syndrome is an autosomal dominant disorder characterized by mild to severe mental retardation and clinodactyly (5th digit). Other symptoms include intrauterine growth retardation and post-natal short stature, developmental delays and difficulties with feeding. We report a case of Coffin Siris syndrome in an 11-year-old Caucasian female with a de novo apparently balanced translocation of chromosome 7 and 22 [t (7;22)(q32;q11.2)] identified by GTG-banding. In addition to our case, there is one other reported case of Coffin - Siris syndrome with a de novo apparently balanced translocation involving chromosome 7q34. Since the breakpoints for both cases are similar this suggests a region location and possibly a candidate region for the Coffin- Siris gene. In the breakpoint region (7q32-34) seen in the two patients, there are several candidate genes: calumenin, podocalyxin-like, prolactin-inducible protein, and eph tyrosine kinase 1, for which no syndrome has yet been associated. Identification of a breakpoint location (7q32) strongly suggest a gene region for the Coffin Siris syndrome.

Program Nr: 1982 from the 1999 ASHG Annual Meeting

Proximal deletion of chromosome 14q. *L. Mehta[1], C. Cervantes[2], K. Small[1], A. Yenamandra[3], P. Koduru[3].* 1) Div. Medical Genetics; 2) Div. Pediatric Endocrinology, Dept. of Pediatrics; 3) Dept. of Laboratories, North Shore Univ. Hospital-N.Y.U School of Medicine, Manhasset, N.Y.

Interstitial deletions of chromosome 14q are rare and are of interest because of the possibility of imprinted genes in this region. We report a patient with deletion 14q13.2-q21.2. The patient was a full term, male infant with birth weight 8.5 lb. He developed severe respiratory distress after birth, eventually resulting in bronchopulmonary dysplasia. He was diagnosed to have congenital hypothyroidism and required progressively increasing thyroid hormone replacement for persistent TSH elevation, despite normalization of thyroxine levels. At 19 months, weight was 10%, height 25% and head size 10%. Motor development was mildly delayed with generalized hypotonia. He had a long face, frontal bossing, downslanting palpebral fissures, high palate and oral hypotonia. Hands and feet were relatively large. No heart defects were present and brain MRI was normal. Karyotype was 46,XY,del(14)(q13.2q21.2).The chromosome analysis was prompted by a case report of chromosome 14q deletion in a child with respiratory and thyroid abnormalities with mild developmental delays (Devrjendt,1998). Proximal 14q deletions are reported to have non-specific dysmorphic features, hypotonia and motor delays. Thyrotrophin abnormalities are described in 2 patients and neonatal respiratory distress in one patient. The latter patient was found to be deleted for PAX9 and TTF-1 (thyroid transcription factor-1) on the paternal chromosome by FISH (Devrjendt,1998). TTF-1 is expressed in embryonic forebrain, lung and thyroid epithelium and regulates production of surfactant B in the lung (Ikeda,1995). Deficiency of TTF-1 could therefore explain both thyroid and respiratory disease. Of note are 3 patients also with similar deletions of 14q13-q21 who had major brain malformations (cebocephaly/holoprosencephaly). These differing phenotypes may be a result of varying extent of deletions or variable expression of deleted genes; however, it would be important to consider parent of origin effects if imprinted genes are present in this deleted segment.

Program Nr: 1983 from the 1999 ASHG Annual Meeting

FISH screening for microdeletions of DMRT1 in 46,XY sex reversed individuals. *A.M. Mengelt[1], C.S. Raymond[2], L.G. Brown[3], D.C. Page[3,4], D. Zarkower[2,5], B. Hirsch[1]*. 1) Department of Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis, MN; 2) Biochemistry, Molecular Biology and Biophysics Graduate Program, University of Minnesota, Minneapolis, MN; 3) HHMI, Whitehead Institute for Biomedical Research, Cambridge, MA; 4) Department of Biology, Massachusetts Institute of Technology, Cambridge, MA; 5) Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN.

Sex reversal is a complex and genetically heterogeneous condition, the etiology of which is unknown in many cases. The role of SRY as a transcription factor and the identification of sex reversed individuals with an apparently intact SRY gene has lead investigators to identify other loci involved in sex reversal. Several X-linked and autosomal loci have been implicated in 46,XY male to female sex reversal, including the distal short arm of chromosome 9, specifically band 9p24.3. Recently, a candidate gene, DMRT1, with homology to the sexual regulatory genes *mab-3* of *C.elegans* and *doublesex* of *Drosophila* was mapped to 9p24.3.

To determine whether microdeletion of DMRT1 is a common mechanism leading to sex reversal, lymphoblastoid cell lines from 27 46,XY individuals with varying degrees of sex reversal were evaluated by FISH with a DMRT1 probe. All cell lines were previously tested for the presence of SRY by PCR, and most were sequenced for mutations in the HMG box domain of SRY.

No deletion of DMRT1 was identified. In a recent study of a larger cohort of such sex reversed individuals, no point mutations of DMRT1 were identified (Raymond et al., 1999). The results of these studies demonstrate that neither deletions encompassing DMRT1 nor point mutations within the coding region of DMRT1 are a common cause of 46,XY sex reversal. Ongoing studies include Southern blot analysis to rule out smaller deletions within DMRT1 that would not have been detected by FISH.

Program Nr: 1984 from the 1999 ASHG Annual Meeting

Cytogenetic internet based external quality assessment pilot study . *J.H. Miyazaki[1], P.R. Wyatt[2], V.M. Lopes[2]*. 1) Laboratory Proficiency Testing Program (LPTP), Toronto, ON, Canada; 2) North York General Hospital, Toronto, ON, Canada.

CytoNet is a Web server for CytoVision users that is within the custody of and administered by Applied Imaging Corporation. This service allows access to cytogenetic digital images for review and interpretation. For twenty-five years, LPTP has provided an annual series of test challenges to evaluate technical and professional proficiency in cytogenetics. Enhancements of the testing model are continually pursued. *Objective: The Laboratory Proficiency Testing Program (LPTP) was to evaluate CytoNet, an Internet enabled case transfer system as to facilitate an external quality assessment program for cytogenetic laboratories. *Study Design: In December 1997, invitations for the pilot study were sent out to the LPTP participants. Four 1998 LPTP surveys were included in the study. Laboratories followed routine procedures to complete each survey. In addition to submitting to LPTP a paper copy of the metaphase(s) and karyotype(s), a digital copy was sent via the Internet using the Applied Imaging CytoNet. Both the paper and digital version of the images were evaluated at LPTP. *Results: Three laboratories submitted their entire case files for the four LPTP surveys. Transmission of all images was successful. The electronic files identified the slides, cells examined and the respective images (metaphase N=120; composite N=16; karyotype N=33 and probe N=13). The paper copy of the images was compared to those submitted by CytoNet. Upon evaluation, minor variation was noted as some of the digital images were deemed to be clearer in resolution than the paper copy. *Conclusion: The use of Internet access to cytogenetic digital images may permit the development of alternate evaluation modalities and a more standardized international external quality assessment system for cytogenetics.

Objective:

Study Design:

Results:

Conclusion:.

Program Nr: 1985 from the 1999 ASHG Annual Meeting

Prenatal diagnosis of GREIG syndrome in a fetus with a de novo translocation t(5;7)(p15;p13). *N. Morichon-Delvallez[1], P. Gosset[1], A.L. Delezoide[1], S. Chemouny[2], S. Loison[1], S. Fert-Ferrer[1], C. Ozilou[1], Y. Dumez[2], M. Vekemans[1], S.P. Romana[1]*. 1) Department of Genetics, Hopital Necker-Enfants Malades, Paris, France; 2) Prenatal Diagnosis Center, Hopital Necker-Enfants Malades, Paris, France.

A 27 years old woman was referred at 27 weeks of pregnancy for amniocentesis because ultrasound examination revealed several anomalies including hydramnios, moderate ventriculomegaly, bilateral syndactyly of the hands and bilateral polydactyly of the feet. Cytogenetic study on amniotic fluid cells in culture showed a de novo apparently balanced translocation t(5;7)(p15;p13). After genetic counselling, the pregnancy was interrupted. Fetopathological examination was carried out and showed the presence of high forehead, postaxial polysyndactyly, moderate ventricular dilatation, abnormal hyppocampus and moderately hypertrophic temporal lobes. The diagnosis of Greig cephalopolysyndactyly syndrome was suggested. A FISH study with a GLI3 probe showed a deletion of the GLI3 locus and confirmed the diagnosis of GREIG syndrome. Firstly this case hightlights the importance of detailed ultrasound examination on cases where an apparently balanced translocation is discovered during prenatal diagnosis. Secondly it shows the importance of FISH studies with locus specific probe to detect breakpoints cryptic deletion associated with de novo chromosome rearrangments.

Program Nr: 1986 from the 1999 ASHG Annual Meeting

Hereditary neuropathy with liability to pressure palsies: two cases with a reciprocal translocation t(16;17)(q12;p11.2) interrupting the *PMP22*gene. *M. Nadal[1], A. Valiente[2], A. Domenech[1], M. Pritchard[1], X. Estivill[1], M.A. Ramos-Arroyo[2]*. 1) Medical and Molecular Genetics, IRO, Barcelona, Spain; 2) Servicio de Genetica, Hospital Virgen del Camino, Pamplona, Spain.

Hereditary neuropathy with liability to pressure palsies (HNPP) or tomaculous neuropathy is an autosomal dominant disease that causes acute or recurrent transient muscle palsies and paresthesias, usually after minor trauma. HNPP is characterized from the pathological point of view by the presence of sausage-shaped swelling of the myelin sheath or tomacula in sensory and motor nerves. A large deletion on the proximal short arm of chromosome 17 was detected in affected members of HNPP. The deleted region is the same as that duplicated in Charcot-Marie-Tooth type 1 (CMT1A). The HNPP phenotype is due to monosomy of the same region in over 85% of cases. The *PMP22*gene spans aproximately 40 kb and contains four coding and two 5-untranslated (1A and 1B) exons. Five mutations in the *PMP22*gene have been described that result in a HNPP phenotype: two frameshift mutations, two nonsense mutations and a splite site mutation. We report here a pedigree with two affected members (mother and son) with HNPP, both of whom carry a reciprocal translocation t(16;17)(q12;p11.2), which we have studied by FISH. The breakpoint on chromosome 17 in both patients lies within exons 1a to 3 of the *PMP22*gene. This is the first case of HNPP being caused by a reciprocal translocation that interrupts the *PMP22*gene. Cytogenetic studies and FISH confirmed the diagnosis of the patient. The two cases presented here not only confirm that the HNPP phenotype was due to the interruption of the *PMP22*gene, but also show the variable penetrance of the phenotype in two related patients carrying the same mutation.

Program Nr: 1987 from the 1999 ASHG Annual Meeting

Unbalanced cryptic t(1q;12p) translocation in an apparently X-linked syndrome with pachygyria, mental retardation and hypogenitalism. *G. Neri[1], E. Rossi[2], C.A. Walsh[3], O. Zuffardi[2], M. Zollino[1]*. 1) Inst Di Genetica Medica, Univ Cattolica, Rome, Italy; 2) Istituto di Patologia Umana ed Ereditaria, Univ di Pavia, Pavia, Italy; 3) Dept of Neurology, Harvard Univ, Boston, MA.

We previously described a new XLMR syndrome with characteristic face, hypogenitalism, congenital hypotonia and pachygyria in 4 members (3 males and one female) of a large family. The trait "pachygyria" was detected in two male patients only, who also presented with a facial appearance typical of the a-talassemia/mental retardation (ATR-X) condition, whose gene, XH2, maps to Xq13.1. Four female carriers were normal, both clinically and neuroradiologically. By linkage analysis with 54 polymorphic X markers, no co-segregation of any allele was observed in all the patients, although a positive lod score (1.701, =0.01) was obtained with marker DXS6673E , mapping to Xq13.1, provided that the male patient without pachygyria was not included. If we consider, indeed, a full penetrance in males of the mutation, this patient should be affected by a different disorder. Mutational analysis of XH2 gene gave normal results. X-inactivation pattern was similarly random in the 4 normal carriers and in the only affected female. From all these clinical and molecular inconsistencies, an umbalanced cryptic translocation was inferred as cause of this condition. FISH analysis with subtelomeric probes, and then with chromosome specific "painting" probes, did show a cryptic t(1;12)(q44;p13.3) translocation:1) a partial 12p trisomy, in association with a partial 1q monosomy, was detected in both patients with pachygyria;2) a partial 1q trisomy, in association with a partial 12p monosomy, was detected in the last two patients without pachygyria. Prometaphase chromosomes were apparently normal, the translocated segments being very similar in size (about 10 Mb) and in banding pattern. These results suddenly make the genetic counseling proper, and the prenatal diagnosis undoubted. In addition, they identify two chromosome regions in which genes involved in cerebral cortical development are likely to reside.

Program Nr: 1988 from the 1999 ASHG Annual Meeting

A simple fluornucleotide coupling method for M-FISH analysis of patients associated with MR and Autism. *M.A. Nimmakayalu, O. Henegariu, P. Bray-Ward, D.C. Ward.* Dept Genetics, Yale Univ, New Haven, CT.

Although 1-2 % of the general population has MR/autism, only few genes responsible for these disorders have been identified to date. Karyotypic changes are detected by Giemsa banding in 10-20% of individuals with mild MR and 40% of those with severe MR and, among individuals with mild to moderate retardation and normal karyotypes, approximately 7.4% have cryptic telomere translocations detectable by LOH analysis. Therefore we began karyotyping a large population of patients with MR, autism and related disorders (currently >100) by two multi-color fluorescence in situ hybridization (M-FISH) techniques: karyotyping using pooled chromosome painting probes that were combinatorially labeled; and telomere analysis using combinatorially-labeled subtelomeric probes. The cost of such screening using commercially available fluor-labeled nucleotides has led us to explore several different methods for coupling fluors to nucleotides or DNA. First, probes were labeled with allylamine-dUTP (AAdUTP) and the DNAs were chemically coupled with N-hydroxysuccinimide esters (NHS) of various fluors or haptenes. However, this two-step labeling procedure was not sufficiently robust for routine work since highly charged fluors gave very weak signals. Next we developed a "non-chemist" approach to rapidly sythesize "semi-pure" dNTPs. AAdUTP is reacted in borate buffer with a NHS of a fluor or haptene. Each reaction was absorbed onto 1 ml of diethylaminoethylyl cellulose (DEAE-52), washed with 0.1 M triethylammonium bicarbonate (TEAB) to remove free fluor or haptene then the fluor or haptene labeled dUTP is eluted with 0.6 M TEAB. The eluates are lyophilzed and resuspended in a Tris buffer. Five to 10 reactions, each at 1-5 umole scale, have been run simultaneously using FITC, CY3, CY5, BIOTIN, DIG, Rhodamine 6G, Texas Red, DEAC, DNP, AMCA. Although these preparations contain unreacted AAdUTP, they work extremely well for probe-labeling. This protocol modification reduces the overall M-FISH probe cost to less than $4/slide.

Program Nr: 1989 from the 1999 ASHG Annual Meeting

Genotype analysis of the Maternal and Paternal Contributions in a 46,XX/46,XY Hermaphrodite. *D. Niu[1], M-y. Chung[2], C-W. Chou[1], B. Hwang[1].* 1) Pediatrics, Veterans General Hospital, Taipei, Taiwan; 2) Medical Research and Education, Veterans General Hospital, Taipei, Taiwan.

True hermaphroditism in human is extremely rare. About 20% of true hermaphrodites show mosaic 46,XX/46,XY karyotype. The mechanisms that cause such conditions are poorly understood. Several mechanisms such as amalgamation of two embryos and parthenogenetic chimeras fertilized by a single or two spermatozoa have been reported previously. All of them have been clearly demonstrated by studies using polymorphic markers. In this report, we describe an infant with true hermaphroditism and a 46,XX/46,XY karyotype in lymphocytes. Nineteen microsatellite markers from eight autosomes and ten from the X chromosome were analyzed in DNAs extracted from the blood of the proband and both parents. At each of the eight fully informative and ten partially informative loci of the autosomes, the proband inherited a single maternal and a single paternal allele. Genotypes at the X chromosome loci also revealed single maternal and single paternal genetic contributions. These results suggest that the mechanism for the 46,XX/46,XY karyotype in this case is different from those reported previously. Hereby, we report this interesting case and possible mechanisms will be discussed.

Program Nr: 1990 from the 1999 ASHG Annual Meeting

Detection of two chromosomal arrangements in the subtelomeric region of 9q in mentally retarded children. *P.T. Nokelainen[1], O. Quarrell[2], B. Kerr[3], R. Regan[1], J. Flint[1]*. 1) Dept. of Molecular Haematology, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, United Kingdom; 2) Center for Human Genetics, Sheffield UK; 3) Royal Manchester Childrens Hospital, Manchester, UK.

One of the major challenges of child neurology is to reveal new causes of mental retardation (MR). 2.5 % of the population suffer from MR (IQ<70): etiological diagnosis is not possible for almost 30% of the moderately to severely affected cases. Subtle chromosomal rearrangments affecting the ends of chromosomes (telomeres) account for about 8% of these cases. Molecular characterization of these small deletions may be a useful tool for the discovery of dosage sensitive genes that give rise to MR and also for an understanding of the processes that give rise to chromosomal aberrations. During a survey of individuals with undiagnosed MR for the presence of subtelomeric rearrangements, two families where identified with abnormalities involving the same region of terminal 9q. In the first family the mother has a balanced translocation between 9q and 13p whilst her two daughters have the unbalanced derivative of chromosome 9, creating a monosomy of the most distal part of 9q. In the second family the affected son has a de novo deletion of the same region. Clinically both girls show severe global developmental delay, profound hypotonia, ligamentous laxity, hearing loss and dysmorphic features. The boy from family 2 shows developmental delay, microcephaly, hypotonia and dysmorphic features. To further characterise these rearrangements, STSs selected from published maps were used to screen PAC libraries. Selected PACs were then used as FISH-probes in order to map the break point regions. The break-point of the first family has been localized to one PAC and the deletion is estimated to be approximately 1.4 Centirays. In the second case the break point was localised more distally. These two deletions represent the smallest known terminal deletions of 9q.

Program Nr: 1991 from the 1999 ASHG Annual Meeting

Abnormal brain developpement and fuzzy chromosomes: a new syndrome? *M. Nouchy[1], E. Gautier[2], C. Fallet[1], N. Bondeux[1], P. Blot[3], C. Nessmann[1], P. Eydoux[1]*. 1) Lab Biol Development, Hosp Robert Debre, Paris, France; 2) Lab Cytogenetique, American Hospital, Paris, France; 3) Obstetrics/Gynecology, Hosp Robert Debre, Paris, France.

We report a patient with cerebral dysplasia and a peculiar aspect of the chromosomes. A severe fetal microcephaly was detected through ultrasound examination, at 37 weeks of an uneventful pregnancy. The parents were not consanguineous, and there was no contributing family history. No other ultrasound abnormalities were seen, and no infectious cause was identified. Chromosome examination from amniotic fluid cells showed a 46,XX chromosome complement. However, an unusual aspect of the chromosomes was noted, with a wavy aspect of the chromatin. Chromatids looked circumvoluted, and G-banding techniques were unsuccessful. Autopsic examination of the fetus showed severe microcrania and microcephaly (-4DS) with no other structural abnormality. Cerebral histology showed a low density of normally differentiated cortical neurons; all other brain structures did not show any abnormality in size or differenciation. A blood karyotype was done in a second center, anaware of the first results. Culture was poor and showed very small, poorly R-banded chromosomes. Parental karyotypes were normal. The patient was pregnant again soon after delivery; a fetal karyotype was performed because of maternal age and did not reveal any abnormality. A normal baby was delivered. This observation could be related to a cytogenetic artefact. However, two independant centers have found a very unusual aspect of our patient's chromosomes.This finding may be related to an abnormal component of the chromatin, altering gene function, and resulting in a possibly autosomal recessive disorder of neuronal growth and/or migration.

Program Nr: 1992 from the 1999 ASHG Annual Meeting

Chromosomal analysis of sperm from severe oligozoospermic men using fluorescence in situ hybridization. *Y. Ohashi, N. Miharu, H. Honda, N. Honda, K. Ohama.* Dept. of OB/GYN, Hiroshima univ. Sch. Medicine, Hiroshima, Japan.

[Purpose]To determine if men with severe oligozoospermia have an elevated risk of aneuploidy in spermatozoa. [Methods] Semen samples were obtained from nine severe oligozoospermic men, six oligozoospermic men and seven normozoospermic men. Routine semen parameters (concentration, motility rate, volume) were analyzed using a Makler counting chamber. Cytogenetic analysis was performed on GTG-banded chromosomes after culture of lymphocytes obtained from peripheral blood. Three color fluorescence in situ hybridization was performed using three probes (D18Z1; biotin and digoxigenin labeled , DXZ1; digoxigenin labeled, DYZ1; biotin labeled) simultaneously. Hybridization and detection was performed basically according to manufacturer's instructions with only minor modification of the post-hybridization washing step. Hybridization signals in sperm nuclei were examined under a Nikon Axiophot microscope to compare aneuploid frequencies of each groups. Statistical analysis was done with chi-square analysis. [Results] Karyotype of all donors were 46, XY. A minimum of 6,000 sperm nuclei per sample for each chromosome were evaluated, for a total of 181,350 sperm nuclei. Hybridization efficiency was 99.8%. Disomic sperm frequencies for chromosome 18, XX, XY, YY were ranged from 0.09 to 0.52, 0.04 to 0.11, 0.05 to 0.59 and 0.04 to 0.14 in men of severe oligozoospermia; ranged from 0.08 to 0.24, 0.03 to 0.12, 0.08 to 0.16 and 0.02 to 0.15 in men of oligozoospermia; ranged from 0.11 to 0.23, 0.06 to 0.19, 0.14 to 0.24 and 0.05 to 0.18 in men of normozoospermia. Diploid sperm frequencies in men of severe oligozoospermia, oligozoospermia and normozoospermia were ranged from 0.20 to1.44, 0.15 to 0.25 and 0.12 to 0.30 respectively. The severe oligozoospermic men showed significantly increased frequencies of XY disomy and diploid spermatozoa comparing to oligozoospermic men and normospermic men . [Conclusions] These data indicate that there might be higher frequency of X-Y non-disjunction in meiosis of spermatogenesis in men of severe oligozoospermia than that in men of oligozoospermia and normozoospermia.

Program Nr: 1993 from the 1999 ASHG Annual Meeting

Hypohidrotic ectodermal dysplasia and Hypomelanosis of Ito in a girl with a de novo t(X;13)(q13;p11.2). *S.J. Orlow[1], R. Marion[2], C. Duncan[3], H. Gu[3], M. Genovese[3], E. Jenkins[3], A. Shanske[2]*. 1) Dermatology, NYU Medical Center, New York, NY; 2) Pediatrics, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY; 3) Institute for Basic Research, Staten Island, NY.

Hypohidrotic ectodermal dysplasia (HED)is caused by mutations of the EDA1 gene at Xq12-13.1 that is critical for the morphogenesis of epidermal derivatives.Heterozygous women may show mild abnormalities of ectodermal derivatives and fully manifesting females look like affected males.Hypomelanosis of Ito(HI)is a heterogeneous disorder characterized by macular hypopigmented whorls, streaks, and patches.Abnormalities of the CNS occur frequently.Mosaicism for different aneuploidies is a common finding and often involves the X chromosome.We evaluated a 5-year-old female with features of both HED and HI.Her physical examination at 4 10/12 years of age revealed an unstigmatized youngster whose height was 104 cm (50%); weight 16 kg (10-25%)and HC 51 cm (50%). She had maxillary hypoplasia,low nasal bridge,conical teeth with increased spacing,periorbital wrinkling,patchy alopecia,and sebaceous hypoplasia over the bridge of her nose consistent with HED and striped hypopigmentation on her trunk following Blaschko's lines consistent with HI.Short term whole blood lymphocyte cultures revealed an apparently balanced de novo X;13 translocation in 40 cells analyzed with a karyotype of 46,X,t(X;13)(q13;p11.2).The same nonmosiac translocation was identified in fibroblast cultures derived from hypopigmented and pigmented areas of skin. Replication X studies using a BrdU terminal pulse showed an inactive normal X chromosome in 100%of blood cells but were unsuccessful in fibroblasts.

We conclude that our patient is a fully manifesting female with HED because the translocation affects the EDA1 gene.Functional disomy has been proposed as a likely mechanism because of the skewed X-inactivation observed in most cases.PCR analysis of DNA extracted from uncultured cells show that a more balanced pattern of inactivation can be observed in these cells.Partial functional disomy in the fibroblast cell lineage may also be responsible for the HI in our patient.

Program Nr: 1994 from the 1999 ASHG Annual Meeting

Chimeric mice containing a human chromosome 21. *M. Oshimura[1], T. Shinohara[1], K. Tomizuka[2], S. Miyabara[3], S. Takehara[1], Y. Kazuki[1], K. Funaki[4], A. Ohguma[2], I. Ishida[2].* 1) Dept Mol Cell Genet, Tottori Univ Sch Life Sciences, Yonago, Tottori, Japan; 2) Pharmaceutical Res Lab, Kirin Brewery Co, Ltd, Takasaki, Gunma, Japan; 3) Dept Pathol, Saga Med School, Saga, Saga, Japan; 4) Dept Biol, Shimane Univ, Fac Education, Matsue, Shimane, Japan.

Since three copies of genes on human chromosome 21 (hChr.21) contribute to Down syndrome (DS), mice containing an additional copy of hChr.21 genes could be a useful model to investigate the contribution of genes or combination of genes responsible for the DS phenotypes. Thus, we attempted to make trans-chromosomic mice with hChr.21 via microcell-mediated chromosome transfer (MMCT). The advantage of using this method is that foreign genetic material could be transferred to mice by using the human chromosome itself as a vector, which enabled us to analyze the expression and function of genes on hChr.21 in the same chromosomal structure as in human. We made chimeric mice using the following steps. (1) We constructed human-mouse A9 monochromosomal hybrids with hChr.21 derived from normal human fibroblasts by whole cell fusion and MMCT. The hChr.21 was tagged with a dominant selectable marker, pSTneo, that can confer G418-resistance to ES cells. (2) The hChr.21 was transferred to mouse TT2F ES cells using MMCT, and the ES cells were injected into 8-cell embryos. We isolated 14 ES clones, and examined the transferred hChr.21 by fluorescence in situ hybridization (FISH) and PCR. 3 of the 15 clones contained an intact hChr.21, whereas other clones contained hChr.21 fragments. 12 chimeric mice exhibiting more than 95% chimerism were produced from 2 of the ES clones with an intact hChr.21. The presence of the transferred hChr.21 was confirmed by PCR and FISH analysis using tail fibroblasts derived from 8 chimeric mice. In 3 of the 8 chimeric mice, the hChr.21 was retained in more than 90% of fibroblasts, although small deletions were present. We are currently investigating (1) the retention and gene expression of hChr.21 in various adult chimeric tissues, (2) germline transmission of hChr.21, (3) phenotypic effects of hChr.21 in chimeric mice.

Program Nr: 1995 from the 1999 ASHG Annual Meeting

Aneuploidy in sperm from fifty-four oligoasthenoteratozoospermic patients undergoing intracytoplasmic sperm injection. *M.G. Pang[1], S.F. Hoegerman[3], J. Pfeffer[2], M. Stacey[3], L. Lunsford[3], C. Osgood[3], G. Donsel[4], S. Oehninger[4], A.A. Acosta[4], W.G. Kearns[3,4,5]*. 1) Biomed. Res. Cent. Korea Advanced Inst. of Sci. and Tech. Taejon, Korea; 2) DHUYS IVF Center, Zerah-Taar-Pfeffer Lab, Bagnolet, France; 3) Cent. for Pediatric Research, EVMS, Norfolk, VA; 4) Jones Inst. for Reproductive Med. EVMS. Norfolk, VA; 5) Inst. Med. Genetics Johns Hopkins Univ. School of Med. Baltimore, MD.

Objective: We summarize data on aneuploidy in sperm from 54 OAT patients undergoing ICSI. **Study Design:** This study determined aneuploidy in sperm from OAT patients and tabulated fertilization and pregnancy rates following ICSI. **Methods:** Aneuploidy frequencies were determined in sperm from 54 OAT patients and 18 proven fertile donors using fluorescence in situ hybridization. Direct labelled DNA specific for chromosomes 1,4,6,8,9,10,11,12,13,17,18,21,X and Y were used and over 200,000 sperm were scored. Chi-square analysis or Fisher's Exact Test were performed. **Results:** Per chromosome disomy frequencies for the gonosomes and autosomes ranged between 0 and 5.7% for patients and 0 to 0.3% for controls. The frequency of diploid sperm ranged from 0 to 9.6% in patients and 0 to 1.2% for controls. The OAT patient group was heterogeneous for the type and frequency of numerical chromosome abnormalities observed. Total aneuploidy in sperm from the 54 OAT patients was estimated to range between 33 and 74%. Total aneuploidy in sperm from the 18 proven fertile donors was between 3.9 and 7.7%. Thirty-six ICSI cycles were performed. Sixty-nine percent (175/252) of oocytes fertilized. Following fresh embryo transfer, 2 preclinical abortion's, 1 first trimester loss and 2 healthy term deliveries (2/26 = 7.7%) resulted. **Conclusions:** There were significant increases in the frequencies of diploidy, autosomal disomy, sex chromosome aneuploidy and total cytogenetic abnormalities in sperm from 54 OAT patients versus controls. The data suggest that meiotic errors occur at highly elevated frequencies in the germ cells of severely affected OAT patients. Males with severe OAT donating sperm for ICSI are at risk of transmitting genetic abnormalities to their offspring.

Program Nr: 1996 from the 1999 ASHG Annual Meeting

UPD risk assessment: Three cytogenetic subgroups. *P.R. Papenhausen[1], J.H. Tepperberg[1], P.N. Mowrey[1], I.K. Gadi[1], H.O. Shah[2], J. Sherman[2], V. Pulijaal[3], H. Nitowsky[3], G. Sachs[3], J.H. Lin[2].* 1) Dept Cytogenetics, Labcorp of America, Res Triangle Pk, NC; 2) Nassau County Med. Center East Meadow, NY; 3) Albert Einstein Col. Medicine Bronx, NY.

Since the recognition of genomic imprinting in man, detection of UPD and determination of its incidence has been crucial for genetic counseling. It is especially important to define predisposing conditions or linked results in prenatal analysis so that appropriate molecular testing can be offered. Starting in early 1996, we have been offering UPD follow up testing for what we felt were risk associated prenatal cytogenetic results; 1) Placental confined mosaicism or single colony amniocyte trisomy, 2)Balanced Robertsonian translocations (RT) involving chromosomes 14 or 15, and 3)Extra marker chromosomes(EAC)derived from chromosome 14 or 15. UPD was detected in all groups, but not in 11 cases with single colony trisomy. One of 12 cases of placental confined trisomy revealed UPD(chr.17). Two cases of UPD were found in the study of 58 Robertsonian translocations. A de novo 13;14 and a de novo 14;14 demonstrated maternal heterodisomy 14 and maternal isodisomy, respectively. The EMC cases generated one additional case of 13 studied (4 de novo). The positive case involved a pedigree with two RT(14;15)mat sib carriers who had balanced carrier fetal analyses with normal biparental molecular results. Their deceased brother had a Prader-Willi clinical diagnosis, the balanced translocation, no deletion, and a chromosome 15 positive bisatellited marker. It was felt this strongly supported UPD 15mat, although molecular confirmation was not possible. Familial RT's may be thought more likely to be associated with UPD than de novo alterations(providing more unbalanced gametes), however, other than the EAC case no UPD was noted in 46 familial RT's studied. One of 12 de novo non-homologous and all three homologous 14;14 RT's had UPD(two archival cases included). It seems likely from a breakaway rescue hypothesis that only denovo EAC's will be associated with UPD. The reported UPD cases appear to confirm the existence of at risk cytogenetic subgroups, although more positive cases are necessary to establish risk.

Program Nr: 1997 from the 1999 ASHG Annual Meeting

The parental origin and chromosomal mechanism for duplication 17p11.2 - the reciprocal recombination product of deletion 17p11.2 associated with Smith-Magenis syndrome. *S.S. Park[1], K.S. Chen[1], L. Potocki[1,2], J.R. Lupski[1,2]*. 1) Dept. of Molecular and Human Genetics; 2) Dept. of Pediatrics and Texas Children's Hospital, Baylor College of Medicine, Houston, TX.

Interstitial duplications of 17p11.2, representing the recombination reciprocal of the common deletion of Smith-Magenis syndrome (SMS), have been found in patients with mild clinical findings such as short statue, behavioral abnormalities and mild mental retardation. We determined the parental origin and chromosomal mechanism of the duplication 17p11.2 in seven dup(17)(p11.2p11.2) patients by fluorescent microsatellite analysis. We used four loci within the common deletion region of SMS, and eleven loci around the region. For quantitative analysis, we compared the height ratios of upper and lower alleles of each genotype with those of the normal control with the same genotype. We found the duplications of the markers within the SMS deletion region in all seven cases, and could elucidate the parental origin and chromosomal mechanism in six cases. Haplotype reconstruction showed that 5 of 6 informative cases are paternal in origin and 1 of 6 is maternal. These data also indicate that this duplication can be derived from either interchromosomal (4/6) or intrachromosomal sister chromatid exchange (2/6). Examining the haplotype of an unaffected sibling to determine the phase of marker genotypes demonstrated unequal crossing over in a patient whose rearrangement occurred by an interchromosomal exchange. These data suggests that duplication 17p11.2 is caused by the homologous recombination reciprocal of the deletion of SMS, and occurs primarily during paternal gametogenesis by either interchromosomal recombination or intrachromosomal sister chromatid exchange.

Program Nr: 1998 from the 1999 ASHG Annual Meeting

Marker gene detection using FISH and CGH in chromosomal abnormality. *Y.J. Park[1], E.K. Park[1], Y.J. Choi[1], D.S. Ko[1], M.H. Park[2], J.S. Park[2], D.W. Kim[2], S.H. Shim[3], Y.H. Cho[3], H.J. Lee[1,2].* 1) Laboratory of Human Genetics, Eulji Med. Science Institute, Eulji Medical College, Seoul, Korea; 2) Department of Physiology, Eulji Medical College; 3) Department of Genetics, University of Hanyang, Seoul, Korea.

We report that two cases of chromosomal abnormality, which one is 47,XY,+mar/46,XY from amniotic fluid of 20 weeks in 35-yr-old woman, the other one is 45,XO,+der(21)/45,XO, from lymphocyte in 23-yr-old woman, detected by using FISH and CGH. In the first case, in order to identify the origin of marker gene which was detected by karyotype, we tried CGH. As a result of CGH, we defined that marker gene is originated from No. of 15 chromosome. And to confirm this finding, we performed the FISH using locus specific gene probe. A result of FISH was demonstrated that this gene is a part of No. of 15 chromosome. Second case was demonstrated as Turner syndrome (45,XO) from karyotype. But, as a result of FISH and CGH, we defined that Y chromosome is translocated in short arm of No. of 21 chromosome. In this case, to determine the gonadectomy, we have to detect expression of Sry gene, which is related with sex determination, from lymphocyte and gonad. In conclusion, it is suggested that FISH and CGH is a more effective technique to define the clinical diagnosis of patients with chromosomal abnormality.

Program Nr: 1999 from the 1999 ASHG Annual Meeting

A mother and two sons with a small terminal rearrangement of Xp leading to a deletion of Xpter-p22.32; A lesson learned. *M.I. Parslow[1], I.P. Miller[1], P.M. MacLeod[2], K.M. Boycott[3], N.T. Bech-Hansen[3].* 1) Cytogenetics Lab, Capital Health Region, Victoria, BC, Canada; 2) Medical Genetics, Capital Health Region, Victoria, BC, Canada; 3) Dept. of Medical Genetics, University of Calgary, AB, Canada.

We present a 45 yr old G7 P3 A4 mother with short stature who has a terminal rearrangement of the short arm of one X chromosome at Xp22.32, and her two sons, who inherited the rearranged X chromosome and who are affected by short stature, mild mental retardation and joint contractures.

Molecular and cytogenetic analysis of one son had demonstrated him to be fragile X negative and with a "normal karyotype". Subsequent to a 22 yr old female sib presenting for reproductive counseling, cytogenetic studies of the mother demonstrated a small terminal rearrangement without any apparent translocation of material to another chromosome. Re-evaluation of the affected son's karyotype, and analysis of the other affected son, demonstrated they both carried the rea(X), but the female sib did not. She was consequently counseled that her pregnancy was not at risk for the familial rearrangement and a healthy normal female baby was born.

FISH to the mother's chromosomes using an X chromosome paint demonstrated hybridization to the complete X chromosome short arm with no hybridization elsewhere in the karyotype. FISH with probes for Kallman syndrome (KAL) and the STS locus demonstrated both these loci to be present on the rearranged chromosome.

This family demonstrates the benefit of maternal studies in shedding light on the presence of subtle X chromosome rearrangements where the family history is suggestive of an X linked condition. It has also permitted further study of the relationship between terminal Xp deletions, proportional short stature, and mental retardation.

Program Nr: 2000 from the 1999 ASHG Annual Meeting

Mosaic chromosomal 22q11.22 deletion. *S.R. Patil, Q. Qian, D. Jordan, K. Horton, W. Rhead.* Division of Medical Genetics, Department of Pediatrics, University of Iowa Hospitals & Clinics, Iowa City, IA.

Mosaicism for obvious autosomal structural abnormalities is rare. Establishing mosaicism with conventional cytogenetics can be problematic, particularly in microdeletion syndromes. We describe here a mosaic case for this deletion. A six-month old female was recently seen in our regional genetics clinic because of minor dysmorphic features and a history of laryngotracheomalacia, peripheral pulmonic stenosis, and declining growth. She was a 3380gm product of a 39 weeks gestation born to a 20 year-old mother. Prenatal and perinatal history was complicated by polyhydramnios, shoulder dystocia and tight nuchal cord and apgars of 2 and 8. She had apnea and choking with feeding, which resolved within a week. At five months, she was below the 5th percentile for height, weight and head circumference. She has small, low-set, over-folded ears, a tented upper lip, a small narrow nose and a small mouth. Neurological examination was normal. Chromosome studies with GTG banding showed a subtle interstitial deletion of the band 22q11.22. FISH studies with TUPLE-1 were done to confirm the deletion. Five out of 55 metaphase cells (9.1%) showed two target signals, and the remaining 50 cells had only one target signal. The existence of mosaicism was confirmed in the repeat blood sample, both in metaphase cells and in interphase nuclei, using the TUPLE-1 and N-25 probes. How common this is at this region of chromosome 22 is not known. It could reflect mitotic instability of this critical region.

Program Nr: 2001 from the 1999 ASHG Annual Meeting

No occurrence of interchromosomal effect in sperm of chromosomal rearrangement carriers evidenced by FISH and PRINS labeling. *F. Pellestor, I. Imbert, P.O. Harmand, B. Andreo.* Inst Genetique Humaine, CNRS, UPR 1142, Montpellier , France.

The possibility that a chromosomal rearrangement might disturb the meiotic behavior of chromosomes not involved in the rearrangement and favour nondisjunction is a contreversial issue in human cytogenetics. An efficient approach to investigate this point is to directly analyse the chromosomal constitution of gametes obtained from carriers of rearrangements. Presently, molecular cytogenetic techniques allow chromosomal analysis on large samples of cell nuclei. The present work was designed to study the occurrence of interchromosomal effect in gametes from rearrangement carriers using FISH and PRINS in parallel. Sperm samples from 4 reciprocal translocation carriers, 1 robertsonian translocation carrier, 1 inversion carrier and 3 control donors were investigated. In each case, dual-color FISH and PRINS procedures were utilized to estimate the incidence of disomy for chromosomes 1, 4, 9, 13, 15, 16, 20 and 21. Among the rearrangement carriers, the frequencies of disomy ranged from 0.08% to 0.34%, comparable to estimates of disomy in control samples. Thus, our results provide no evidence for the occurrence of a meiotic interchromosomal effect in male carriers of single balanced rearrangements.

Program Nr: 2002 from the 1999 ASHG Annual Meeting

Narrowing the 4q- syndrome critical region to the band 4(q32-33). *C. Perandones[1], M. Segovia[1], A. Corominas[1], E. Pastene[1], M. Yannibelli[2], R. Bennun[2], O. Pivetta[1].* 1) Clinical Genetics Dept., Natl Ctr of Medgenetics, Buenos Aires, Argentina; 2) Asociacion Piel, Buenos Aires. Argentina.

Interstitial deletion of the long arm of chromosome 4 is a relatively rare anomaly and is classfied into 2 o3 clinically distinct entities: the distal interstitial and terminal (4q33-qter) deletions and the more proximal (4q21-q22) ones. The most commonly reported rearrangement of 4q is deletion 4q31 which is associated with a characteristic phenotype of mental and growth retardation , absent glabella, upward slanting palpebral fissures, hypertelorism, cleft lip and palate, micrognathia, abnormal ears and unique pointed 5th finger which is found in more than two thirds of patients. One patient with an intersititial deletion from band 4q31.22 :: 4q34.2(Sarda et al,1992) with the full phenotype suggested the critical region for the 4q- syndrome phenotype was delimited by these breakpoints. We report on a child with a small interstitial deletion within band 4q32 who exhibits most of the features of the established 4q phenotype. The proband is a 3 year-old boy with microcephaly (-2DS), mental and growth retardation, upward-slanting palpebral fissures, hypertelorism, short nose with flat nasal bridge, cleft lip and palate, micrognathia, abnormal ears, epicanthus, long eyelashes, altered palmar flexion creases and severe bilateral hydronephrosis with hydroureter and vesicoureteral reflux. Chromosomal analysis by G banding to the level of 550 bands per haploid genome demonstrated 46,XY,del(4)(q32-33). Our patients phenotype would imply that most of the components of the 4q- syndrome including the characteristic dysmorphism mainly results from loss of the segment q32.q33. This case together with those previously quoted suggests that the 4q- phenotype is not restricted to terminal deletions and that the severity of the phenotype is not necessarily proportional to the extent of the missing segment.

Program Nr: 2003 from the 1999 ASHG Annual Meeting

Comparative Mapping of Cercopithecus aethiops by karyotyping, chromosome painting and M-FISH. *M.J. Pettenati, C. von Kap-Herr, M. Saner.* Dept Pediatrics/Div Med Gen, Wake Forest Univ Sch Medicine, Winston-Salem, NC.

The Old World monkey family Cercopithecidae includes macaques and the African green monkey (Cercopithecus aethiops). Both species play important roles in biomedical research. Macaques and C. aethiops are not closely related, however, physically they are very similar. Cytogenetically, macaques are 2n=21 while C. aethiops are 2n=30. Karyotypic comparisons have suggested that the C. aethiops karyotype could be reconstructed after fusion to match the macaques. The banding pattern homology between C. aethiops and humans is believed to be nearly 95% although chromosome banding for chromosome homology is considered subjective.

To determine C. aethiops synteny, we used karyotyping, chromosome painting and M-FISH. Blood from 16 C. aethiops were cultured using standard cytogenetic techniques. Chromosomes were GTG. A total of 15 human chromosome paints were used initially per manufacturer's protocols with the exception of a 68-hour hybridization. M-FISH was performed using the VYSIS SpectraVysion Assay. Protocol modifications included a reduced stringency wash of 50% formamide/1XSSC at 40^{o}C.

Chromosome analysis of C. aethiops revealed a modal number of 60. Karyotypic comparison of C. aethiops to macaques and humans suggested that nearly all C. aethiops chromosomes have a similar banding pattern when considering fission as an evolutionary event. Karyotypic analysis suggests that C. aethiops arose from macaques as a result of 7 fission events. Human chromosome paints and M-FISH identified 6 fission and 1 fusion event. M-FISH was most useful in delineating the complete C. aethiops/macaque/human chromosome synteny.

This data showed agreement with 7 of the reported chromosome homologies and identified 16 new chromosomal homology assignments. Only those chromosomes homologous to human chromosome 2 are still present as separate chromosomes in both macaques and C. aethiops supporting the concept that formation of a single human chromosome was a major step in human evolution. This study delineates the complete chromosomal homology between C. aethiops, macaques and humans.

Program Nr: 2004 from the 1999 ASHG Annual Meeting

Isolation of the motile fraction does not separate aneuploid from haploid sperm prior to intracytoplasmic sperm injection (ICSI). *J. Pfeffer[1,2], M.W. Stacey[1], M.G. Pang[3], S.F. Hoegerman[1], L. Lunsford[1], G. Doncel[4], A.A. Acosta[4], W.G. Kearns[1,4,5]*. 1) Center for Pediatric Research, EVMS, Norfolk, VA; 2) DHUYS IVF Center, Bagnolet, France; 3) Biomed Res Cent, Korea Adv Inst of Sci and Tech, Taejon, Korea; 4) Jones Inst EVMS, Norfolk, VA; 5) Inst of Genet Med, Johns Hopkins Univ Sch of Med, Baltimore, MD.

Objective: We summarize data on aneuploidy in sperm from 23 infertile males with oligoasthenoteratozoospermia (OAT) undergoing ICSI. **Design:** Prospective study to determine whether isolation of the motile fraction separates aneuploid from haploid sperm. **Materials and Methods:** FISH to determine aneuploidy frequencies for chromosomes 1, 13, 18, 21, X and Y in sperm from swim-up and pellet fractions. **Results:** Chromosome aneuploidy in patients was significantly ($p < 0.05$) greater versus controls. Per chromosome disomy for patients ranged between 0.0 and 3.8% in swim up and 0.0 to 2.1% in pellets; for controls, the frequencies were 0.0 to 0.3% in swim up versus 0.0 to 0.7% in pellets. The frequencies of diploid sperm in patients were 0.0 to 1.7% in swim up versus 0.0 to 1.4% in pellets; for controls the rates were 0.0 to 0.3% in swim up versus 0.3 to 1.1% in pellets. Per chromosome aneuploidy for patients ranged from 0.0 to 10.0% in swim up versus 0.3 to 3.9% in pellets; in controls the rates were 0.0 to 0.6% in swim up versus 0.0 to 0.8% in pellets. Total aneuploidy in sperm from whole semen ranged between 32 and 69%. Patient-to-patient heterogeneity was shown. **Conclusions:** The data show significantly higher rates of diploidy, autosomal disomy and nullisomy, sex chromosome disomy and nullisomy and total aneuploidy in sperm from all separated fractions from all OAT patients versus controls. The type and percent of aneuploid sperm for all OAT patients studied found in both swim-up and pellet fractions was not different, with the exception of diploid sperm, which remained in the pellet fraction. Isolation of the more motile sperm fraction does not separate aneuploid from haploid sperm in these patients. These infertile males may be at an increased risk of transmitting genetic abnormalities to their offspring.

Program Nr: 2005 from the 1999 ASHG Annual Meeting

Is pediatric T-cell ALCL always associated with an ALK rearrangement? *D. Pickering[1], P. Aoun[2], D. Weisenburger[2], M. Wiggins[1], W.G. Sanger[1].* 1) Human Genetics, Univ Nebraska Med Ctr, Omaha, NE; 2) Pathology/Microbiology, Univ Nebraska Med Ctr, Omaha, NE.

Anaplastic large cell lymphoma (ALCL) is a distinct subtype of NHL morphologically characterized by large pleomorphic tumor cells which express CD30 antigen. It is predominantly seen in children and young adults and has a better overall survival than other large cell lymphomas. These tumors are typically T or null immunophenotype and have variable histological features. A specific genetic subtype of this heterogeneous group is characterized by a 2p23/ALK rearrangement which is most often seen as the t(2;5)(p23;q35)-associated NPM/ALK fusion. This abnormality has been reported in up to 50% of all ALCL; however, recent studies have shown a molecular variant of this rearrangement which can be cytogenetically indiscernible. The focus of this study was to examine all pediatric T-cell ALCL's studied at our institution and to compare the cytogenetics, FISH and immunohistochemistry of these tumors. There were 17 ALCL cases and 11 of them had cytogenetics and/or FISH data available for this study. All 11 cases were CD30+ and positive for the ALK protein by immunostaining. Seven of the 11 cases were positive for the t(2;5)(p23;q35) and FISH studies were concordant with these results. Three cases did not have cytogenetics but were positive for the ALK rearrangement by interphase FISH studies on paraffin block tissue. One case was t(2;5) negative but exhibited NPM/ALK co-localization in two different complex derivative chromosomes which were ascertained by FISH and M-FISH procedures. These findings suggest that all ALCL of T-cell origin cases in this series exhibited aberrant ALK activity and that correlation of the cytogenetics, FISH and immunohistochemistry very specifically identifies this unique genetic subtype of NHL. This study demonstrates that even though classical cytogenetics may not reveal a t(2;5) in all cases, ALK FISH and extended FISH procedures (i.e., M-FISH) can resolve the variant ALK rearrangement which occur with this disease. Supported in part by the National Childhood Cancer Foundation.

Program Nr: 2006 from the 1999 ASHG Annual Meeting

A complex chromosome rearrangement in a patient with multiple anomalies. *L. Plumley, J. Yu, A. Szekely, B. Pober, T. Yang-Feng.* Genetics, Yale University Med. School, New Haven, CT.

The proband was a 37 week female product to a 22 yr old primigravida. Family history was unremarkable. Prenatal ultrasound revealed IUGR. Birth weight was at the 3rd centile, length and head circumference were at 15-25th centiles. Dysmorphic facial features including a round face with upslanting palpebral fissures, large nose with a flattened bulbous tip, U-shaped cleft palate and mild micrognathia. She also had a sacral dimple, joint laxity, transverse palmar creases, proximally set, finger-like thumbs, arachnodactyly of fingers II-V, irregular toes, and bifid 5th toes. She was irritable but otherwise had normal tone and newborn reflexes. Further evaluations revealed retinal detachment with normal brain and spinal cord imaging.

Cytogenetic study revealed a complex chromosome rearrangement including a translocation between chromosomes 5 and 8, a der(16) resulting from a translocation between chromosomes 11 and 16 and a rearranged chromosome 11 which cannot be fully delineated. Both parents are chromosomally normal. FISH study using whole chromosome painting probes confirmed these abnormalities and demonstrated that both ends of the abnormal chromosome 11 showed positive hybridization signals for the chromosome 16 probe. This rearranged chromosome 11, thus, appears to have arisen from a translocation between the long arms of chromosomes 11 and 16, followed by a duplication of 16q material which was then moved to distal 11p after an inversion. During this process, an interstitial deletion of 11q and possibly 11p occurred. The patient thus has partial deletion of 11q (and ?11p) and partial duplication of 16q. Her phenotypic abnormalities have some overlap with the del(11q) syndrome. The patient's karyotype can best be designated as: 46,XX,t(5;8)(p15.3;p23), der(11)(16qter®16q22::11p15®11q?14::16q22®16qter),der(16)(16pter®16q22::11q23®11qter). Further characterization of this unique rearrangement with chromosome region-specific probes is in progress.

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Program Nr: 2007 from the 1999 ASHG Annual Meeting

Molecular cytogenetic evaluation of a de novo balanced translocation associated with Blepharophimosis, Ptosis and Epicanthus inversus Syndrome. *V. Praphanphoj[1,2], G.H. Thomas[1,2], B.K. Goodman[2,3], M.J. Dixon[4], C. Toomes[4], K. Nield[4], M.T. Geraghty[1]*. 1) Dept. Pediatrics, Johns Hopkins Hospital, Baltimore, MD; 2) Kennedy-Krieger Institute, Baltimore, MD; 3) Dept. Gynecology and Obstetrics, Johns Hopkins Hospital, Baltimore, MD; 4) School of Biological Sciences, University of Manchester, Manchester, UK.

Blepharophimosis, ptosis, epicanthus inversus syndrome type I (BPES; OMIM 110100) is an autosomal dominant disorder affecting craniofacial development and ovarian function. Recent studies in a patient with a balanced translocation [46,XY,t(3;4)(q23;q15.2)] assigned the critical region to a 45 kb interval on chromosome 3q23. We have identified a patient with BPES who presented in the newborn period with characteristic facial features. Chromosome analysis showed a de novo reciprocal translocation involving chromosome 3q23 [46,XX,t(3;21)(q23;q22.1)]. FISH studies were performed using PACs 108L15 and 106C10 (RPCI1) and a BAC 175G20 (Research Genetics) as probes. These clones were previously shown to map to 3q23 and to cross the breakpoint in the first patient described above. In FISH studies on our patient, each of the above clones simultaneously hybridized to both derivative chromosomes. This confirms that the breakpoint in our patient also resides within the overlapping region of the three clones. Using BAC 106C10 as a probe, we observed an unequal signal intensity on the derivative chromosomes, with the weaker signal being present on the der(3). Combining these results with the previous case we estimate that the breakpoint in our patient lies within 60 kb of the centromeric end of BAC 106C10. To our knowledge, this is the second patient with BPES and a balanced translocation that has been evaluated by molecular cytogenetics. Further studies in this region will help to elucidate the gene defective in this disorder.

Program Nr: 2008 from the 1999 ASHG Annual Meeting

Reciprocal 1;17 translocation in a patient with apparent Aicardi syndrome plus complex congenital heart disease and limb malformation. *M.C. Prieto[1], S. Khan[1], C. Tuck-Muller[1,3], S. Li[1,3], R. Gordon[2], M. Marble[1]*. 1) Human Genetics Program, SL31, Tulane Univ Medical Ctr, New Orleans, LA; 2) Department of Ophthalmology, Tulane University Medical Ctr, New Orleans, LA; 3) Department of Medical Genetics, University of South Alabama, Mobile, AL.

We present a 3-month-old girl with microphthalmia, chorioretinal lacunae, vertebral malformations, total agenesis of the corpus callosum, Dandy Walker anomaly, EEG abnormalities, limb defect and congenital heart disease (single left ventricle with AV canal). Most of these findings are indicative of Aicardi syndrome, an X-linked disorder mapping to Xp22. However, congenital cardiac anomalies and limb abnormalities are not part of Aicardi spectrum. Chromosome analysis revealed an apparently balanced *de novo* reciprocal 1;17 translocation. C-banding confirmed the breakpoint in chromosome 1, and the heterochromatic band of chromosome 1 was split between the derivative 1 and the derivative 17. Fluorescence *In Situ* Hybridization (FISH) using DNA probes (from Oncor, Inc.) specific for Smith-Magenis (D17S258) and Miller-Dieker (D17S379) regions confirmed the breakpoints in chromosome 17. The STS probe (Oncor, Inc.), specific for the steroid sulfatase region (Xp22.3) which maps close to but distal to the Aicardi locus, hybridized to both X chromosomes. The karyotype was determined to be 46,XX,t(1;17)(q12;p12).ish t(1;17)(q12;p12) (D17S379+;D17S258+,D17S379-),Xp22.3(STSx2). In conclusion, our patient has the typical features of Aicardi syndrome but also has a complex congenital heart disease and a limb abnormality. We speculate that the apparently balanced 1;17 translocation may have a submicroscopic imbalance causing Aicardi-like features with additional anomalies. Alternatively, our patient may have Aicardi syndrome due to an X-linked mutation and the heart and limb anomalies due to the translocation.

Program Nr: 2009 from the 1999 ASHG Annual Meeting

Predominance of Down syndrome phenotype in mos 45,X,+mar(X)[18]/46,XX,+21,der(21;21)(q10;q10)[2] in cardiac tissues. *H.H. Punnett[1], A.S. Schneider[2], D.W. Sabol[3], T.M. Bardakjian[2], K.A. Martin[2], P.N. Howard-Peebles[4], P.K. Rogan[5]*. 1) St Christopher's Hosp Children, Philadelphia, PA; 2) Albert Einstein Medical Center, Philadelphia PA; 3) Phylogenetix Laboratories, Pittsburgh PA; 4) Genetics & IVF Institute, Fairfax VA; 5) The Childrens Mercy Hospital and Clinics, Kansas City MO.

We report an infant with de novo mos 45,X,+mar(X)/46, XX, +21, der (21;21)(q10;q10). Although clinical features of both Down and Turner syndromes are usually evident in patients with this karyotype, only findings of Down syndrome have been noted. Endocardial cushion defect and short femurs were seen on ultrasound. Duodenal atresia and Down syndrome stigmata were documented postnatally. In amniocytes, 46, XX, +21, der (21;21)(q10;q10) was twice as prevalent as 45,X, +mar(X). Similar proportions of each cell line were evident in cord and peripheral blood. Surprisingly, chromosomal studies of cardiac tissues (atrium, ventricle, and connective) obtained during surgery revealed ~10% with 46,XX,+21,der(21;21) and ~20% in pericardium, whereas both cell lines were equally represented in thymus. This suggests that cardiac findings in Down syndrome are the result of a specific subset of cells harboring trisomy 21, rather than being based on the proportion of cells with this karyotype. The intact sex chromosome in the 45,X,+mar(X) line was maternally derived, based on a comparison of thymus and cardiac tissue genotypes. The parental origins of the derivative 21 and the marker could not be determined due to the small size of the marker chromosome and presence of only two chromosome 21 alleles at each locus analyzed. Two alleles were present by microsatellite analyses of markers on each of 6 euploid chromosomes (excluding X and 21), suggesting that mixed aneuploidy resulted from simultaneous loss of both chromosomes rather than chimerism. These distinct abnormal cell lines presumably arose prior to the morula stage of development because both karyotypes were observed in the embryoblastic and extraembryonic mesodermal lineages.

Program Nr: 2010 from the 1999 ASHG Annual Meeting

Complex Chromosome Rearrangement (CCR) involving chromosomes 2, 5, 11, and 16 and a reciprocal translocation between chromosomes 6 and 8 verified by spectral karyotyping (SKY) in a girl with mild dysmorphic features and mental retardation. *E.S. Ramos[1], S.R. Rogatto[2], L. Martelli[1,3], A. Piram[1], S.A. Santos[1], J.A. Squire[4]*. 1) Dept.of Genetics, School of Medicine of Ribeirao Preto, University of Sao Paulo, Rib. Preto, SP, Brazil; 2) Dept. of Genetics, Instituto de Biociencias, UNESP, Botucatu, SP, Brazil; 3) Div. of Genetics, Sinha Junqueira Maternity Foundation, Rib. Preto, SP, Brazil; 4) Ontario Cancer Institute, Princess Margaret Hospital, University of Toronto, University of Toronto, ON, Canada.

Congenital Complex Chromosome Rearrangements (CCRs) compatible with life are rare in humans. We report an apparently de novo CCR involving chromosomes 2, 5, 11, and 16 and a reciprocal translocation between chromosomes 6 and 8, in a 4 1/2-year-old girl. Clinical features associated were mild dysmorphic features and psychomotor retardation. The proband was born to a 29-year-old father and a 19-year-old (G4P4A0) mother at 37 weeks of gestation. No consanguinity was related. Birth weight was 3110g and length was 48cm. She was reffered to us at the age of 4 1/2 years and her weight was 18000g (p50-75), length was 97.5cm (p3-10) and head circunference was 51.5cm (p50). The clinical examination showed apparent telecanthus [but the inner canthal distance was 3.2 cm (p75-90)], infiltrated upper eyelids, broad nasal root, depressed nasal bridge, long philtrum, large mouth, thin upper lip, spaced teeth with maxillary midline diastema, dysplastic ears, short neck, umbilical hernia and Sydney line on the left hand. There was neither visceral (cardiac, abdominal or pelvic) malformation nor anomaly in standard laboratory investigations. Conventional cytogenetic analysis on peripheral blood lymphocytes revealed a CCR in 100 metaphases. Spectral karyotyping (SKY) was performed as an adjunct to high-resolution GTG-banding for characterization of the abnormal chromosomes. The final karyotype was 46,XX,t(6q;8p),t(2q;5q;11q;16q). We point out the usefulness of SKY in elucidating CCRs and other cryptic constitutional chromosomal changes.

Supported by FAEPA, FAPESP, NCIC and CCS.

Program Nr: 2011 from the 1999 ASHG Annual Meeting

Cardio-facio-cutaneous syndrome phenotype in an individual with an interstitial deletion of 12q. *K. Rauen[1], S. Bitts[2], P. Cotter[1,2], V. Cox[1], M. Golabi[1]*. 1) Department of Pediatrics-Division of Medical Genetics, University of California San Francisco, San Francisco, CA; 2) Division of Medical Genetics, Children's Hospital Oakland, Oakland, CA.

Cardio-facio-cutaneous syndrome (CFC) and Noonan syndrome (NS) are thought to be allelic or part of a contiguous gene syndrome. The molecular basis of both disorders is unknown. Only one patient with a cytogenetic abnormality and a Noonan-like phenotype has been reported in which an interstitial deletion of proximal 12q was identified: del(12)(q12q13.12). However, more recently, families have been reported with individuals having phenotypes characteristic of both NS and CFC suggesting variable expression of the same genetic locus. In one such family, linkage analysis has mapped the NS/CFC region to 12q24. We describe a 19 month female with features of CFC including developmental delay, growth retardation, macrocephaly, abnormal cranial contour, ptosis, telecanthus, blepharophimosis, short nose, depressed nasal bridge, bulbous tip, small nares and a small chin. Skeletal findings include pectus excavatum and a broad chest with wide-spaced hypoplastic nipples. Ectodermal abnormalities include fine, sparse hair, scant eyebrows and generalized hyperkeratotic papules. Brain MRI revealed significant delay in myelination with an overall reduction in white matter volume and hydrocephalus. Karyotyping of peripheral blood lymphocytes showed an interstitial deletion of one chromosome 12 in all metaphases examined: 46,XX,del(12)(q21.2q22). FISH analysis was performed with microdissected chromosome band specific probes for 12q21 and 12q22-23. The del(12) chromosome showed a significant reduction in the size of the hybridizing region for the 12q21 probe, confirming that the chromosome band 12q21.3 was deleted. CFC may represent a heterogeneous disorder. Individuals with a severe phenotype, as in our patient, may have a 12q21.3 deletion, whereas a milder phenotype may be due to a microdeletion. This is suggestive of a contiguous gene syndrome.

Program Nr: 2012 from the 1999 ASHG Annual Meeting

A stable acentric marker chromosome formed by interstitial deletion of 17q and subsequent inverted duplication of the deleted segment resulting in partial trisomy for 17q22 to 17q23 diagnosed in a dysmorphic newborn. *J.B. Ravnan[1], K. Ouellette[1], A. Fabre[1], D.C. Crenshaw[1], S. Guillory[2], R. Siewert[2], S. McCoy[2], J. Kothari[2].* 1) Genzyme Genetics, Santa Fe, NM; 2) Provena Saint Joseph Hospital, Elgin, IL.

A 34 year old G4P2/0/1/2 Hispanic female gave birth to a baby boy at 38 weeks gestation. Her history was significant for an IUFD at 32 weeks gestation. Prenatal ultrasound and maternal serum alpha fetoprotein were reported as normal. At birth, a small placenta and a bifurcation at the base of the umbilical cord of 1 cm in length were noted. Initially the baby was hypotonic and had Apgars of 3, 6, and 10. Dysmorphic features noted at birth include a sacral dimple with a hair tuft over the base of the spine, low set ears, high arched palate, bilateral undescended testes, slight hypotonia, high pitched cry, large open anterior fontanel, wide split between the big and second toe, and syndactyly bilaterally between the 2nd and 3rd toes. A newborn metabolic panel, echocardiogram, and renal ultrasound were normal. The baby was discharged home at 6 days.

Chromosome analysis revealed a male karyotype with an interstitial deletion of the long arm of chromosome 17 and an additional marker chromosome in every cell examined. Fluorescence in situ hybridization (FISH) studies demonstrated that the marker chromosome was derived from inverted duplication of the deleted segment of chromosome 17 and did not contain detectable centromeric alpha-satellite sequences. The resulting karyotype was interpreted as 47,XY,del(17)(q22q23),+der(17)(q22->q23::q23->q22). This results in partial trisomy for 17q22 to 17q23. This report provides another example of a newly-defined class of mitotically stable marker chromosomes which lack alpha-satellite sequences.

Program Nr: 2013 from the 1999 ASHG Annual Meeting

Subcortical white matter abnormalities and rib anomaly in a child with Trisomy 12p syndrome. *P.J. Reitnauer[1,2], W.O. Young[1], C.M. Powell[2], M.J. Pettenati[3].* 1) Pediatric Teaching Program, Moses Cone Health System, Greensboro, NC; 2) Pediatrics and Medical Genetics, UNC, Chapel Hill, NC; 3) Medical Genetics, WFUMC, Winston-Salem, NC.

Trisomy 12p syndrome is characterized by typical craniofacial features and mental retardation. We are following a child diagnosed as a neonate with trisomy 12p as a result of a paternal reciprocal translocation involving chromosomes 12 and 14 [46,XX,-14,+der(12)t(12;14)(q11;q11.2)pat]. Our patient has dysmorphic facies, post-axial polydactyly and global delays. Nystagmus was apparent in infancy and adduction of each eye is limited. There is no gross visual impairment. The optic nerves and retinas are clinically normal. There have been no seizures. Motor development is progressing with a 6 month delay. The head circumference is at the 10th percentile at age 14 months (weight and length just below the 5th percentile). Clinical features in our patient that have not been previously described include persistent direct hyperbilirubinemia of unknown cause which resolved, absence of the left 12th rib and dextroposition of the heart with normal anatomy. Magnetic resonance imaging of the brain showed extensive patchy abnormal signal throughout the deep and subcortical white matter of both hemispheres. Other areas of myelination appeared normal for age. The corpus callosum was normally formed. There was a small choroid fissure cyst. It has been suggested that a dosage effect of genes on chromosome 12p may play a role in brain development given the findings in other conditions associated with chromosome 12p duplications such as Pallister-Killian syndrome (tetrasomy 12p) and acrocallosal syndrome (12p13.2-p11.2 inverted tandem duplications, agenesis of the corpus callosum, seizures and post-axial polydactyly). A recent report by Elia et al.(1998) postulated that the high signal abnormalities seen on the brain MRI of their patient with trisomy 12p could have been caused by a perinatal event. The presence of similar findings in our patient supports the possibility that dysmyelination could be a significant feature of trisomy 12p syndrome and associated with neurodevelopmental gene(s) on chromosome 12p.

Program Nr: 2014 from the 1999 ASHG Annual Meeting

Multiple applications of FISH analysis with locus-specific probes generated by means of a novel procedure. *P. Riva, L. Corrado, A. Bentivegna, P. Colapietro, L. Larizza.* Dept Biol & Genetics, Univ Milan, Milan, Italy.

The availability of suitable locus-specific (LS) probes for FISH analysis improves both diagnosis and basic science making the characterization of chromosomal breakpoints rapid and precise. The current way of generating LS probes involves the stepwise subcloning of clones with large inserts and/or screening of genomic libraries. As alternative to this approach we set up a novel procedure which, by selection of LS sequences from YAC clones, allows to produce LS probes. It involves single strand DNA polymerization by a specific primer on YAC DNA and subsequent combined locus-specific-Alu PCR. Gel electrophoresis of labeled single strand DNA allowed to assess that the most represented DNA fragments ranged from 0.6 to 5 Kb. The use of two LS primers extending the polymerization toward opposite directions allows to obtain a product, even at a low yield, covering about 10 Kb around the selected locus. The subsequent locus-specific-Alu PCR on single strand DNA greatly enrich the selected genomic region making it suitable to be labeled for FISH analysis. We apply this novel procedure to remove the non desirable signal of chimeric YACs and to obtain a refined characterization of gross deletions of NF1 patients. The resolution of this procedure was assessed by using an NF1 intron-27-specific probe which allowed a small intragenic deletion to be detected. Sixteen LS probes developed from 17q11.2 contig YACs also allowed SUPT6H, BLMH and ACCN1 genes to be precisely mapped within gross deletions of NF1 patients and deletion boundaries to be identified in each case. The procedure also allows the generation of FISH probes from ESTs or cDNA sequences without undertaking time-consuming experiments. We successfully used LS probes for fiber FISH experiments showing that this kind of probes is suitable for high resolution mapping of closely linked loci. Some of the inconveniences that hamper LOH testing in tumors, such as the need of polymorphic markers, may be overcome by this method. FISH test of LOH can provide information on heterogeneity of tumor cells facilitating identification of critical homozygous LOH regions. Patent number MI98A 002060. Supported by Telethon.

Program Nr: 2015 from the 1999 ASHG Annual Meeting

Partial hexasomy of 15q11-q13 in a child with two supernumerary inv dup(15) chromosomes. *C. Sarri[1], J. Gyftodimou[1], H. Laskari[2], M. Grigoriadou[1], E. Pandelia[1], M.B. Petersen[1].* 1) Dept Genetics, Inst of Child Health, Athens, Greece; 2) 1st Pediatric Clinic, "Aghia Sophia" Children's Hosp, Athens, Greece.

Inv dup(15) is the most common supernumerary marker chromosome in humans. An eight month old female infant was referred to our Dept. because of severe psychomotor retardation, cleft lip/palate and hypotonia. Cytogenetic investigation showed that she carried two extra bisatellited chromosomes in all cells. Fluorescent in situ hybridisation (FISH) with a chromosome library 15 probe identified the supernumerary chromosomes as inv dup(15). FISH using the SNRPN probe mapping to the Prader-Willi/Angelman syndrome (PW/AS) region implied that each extra chromosome contained two copies of the PW/AS critical region. Molecular analysis revealed biparental inheritance for the two normal chromosome 15 homologues (i.e. no UPD). To our knowledge, our case is the second in the literature having the PW/AS region six times in each cell examined. Since most individuals carrying a small inv dup(15) are asymptomatic, it seems that the symptomatic ones are associated with an increased dosage of the PW/AS region.

Program Nr: 2016 from the 1999 ASHG Annual Meeting

Prenatal diagnosis of a stable *de novo* dic(13;18)(p12;p11.3) with deletion of 18p subtelomeric sequences using chromosome and FISH analysis with postnatal follow-up. *M.A. Scanlan-Stahl[1], H.D. Dietrich-Cook[1], J. Wailand[1], J.E. Takacs[1], S.E. Mundt[2], J.B. Ravnan[1]*. 1) Cytogenetics Laboratory, Genzyme Genetics, Santa Fe, NM; 2) Perinatal Center, Saint Luke's Hospital of Kansas City, Kansas City, MO.

An amniocentesis was done for a 36 year old G3P2 woman after a maternal serum screen gave an increased risk for Down syndrome (1/109). Chromosomal analysis revealed a stable dicentric translocation, 45,XY,dic(13;18)(p12;p11.3), that appeared balanced at the 400 band level. Fluorescence *in situ* hybridization (FISH) studies showed the presence of the chromosome 13 centromere and rRNA genes and deletion of the 18p subtelomeric region. Dicentric chromosomes may be stable or unstable during cell division; chromosomes studied from cord blood confirmed the dicentric rearrangement to be stable. Parental blood chromosome studies indicated normal chromosomes. Prenatal ultrasound at 34 weeks showed a two vessel cord, although a second trimester ultrasound had documented a three vessel cord. The pregnancy was delivered by induction at 38 weeks. The two vessel cord, a left club foot (also seen in a paternal cousin) and a suspected subtle urogenital anomaly were noted. The baby resembled his siblings at birth, and at three months of age he shows normal development.

Program Nr: 2017 from the 1999 ASHG Annual Meeting

Cytogenetic and molecular characterization of evolutionary chromosome breakpoints in great apes. *Z.H. Shan, E. Zend-Ajusch, F. Gruetzner, T. Haaf.* Max-Planck-Institute for Molecular Genetics, Berlin, Germany.

While differences between man and great apes are obvious, they need not have involved a large number of adaptive changes in gene loci. Most of the genome remained relatively undisturbed. Most current models postulate a secondary role for chromosome rearrangements in speciation by their acting as a postmeiotic barrier between closely related species. However, it is also possible that evolutionary chromosome rearrangements are functionally important. They may interfere with genome function either directly, by disrupting a gene(s), or indirectly, by (in)activating closely juxtaposed genes by position effect. To address this question, we performed a systematic search for genes in evolutionary breakpoint regions. FISH with genetically and cytogenetically anchored YACs was used to visualize homologous regions on great ape chromosomes and to characterize evolutionary chromosome rearrangements in fine detail. In particular, we have studied a translocation in the gorilla lineage involving the GGO homologs of HSA 5 and 17. We identified breakpoint-spanning YACs for the two breakpoint regions in HSA 5q13 and 17p12. Alu-PCR products of these YACs were used to isolate smaller genomic clones, such as PACs and cosmids, which were then used to build contigs of the breakpoint regions. cDNA library screening, exon trapping, and Alu-splice PCR strategies are being used to isolate genes from the breakpoint regions. Thus far, we have mapped and characterized the human homolog of a mouse gene which may be involved in intracellular signaling. It is well known that pericentric inversions in PPY 2 (homolog of HSA 3) separate the Borneo and Sumatra orangutan subspecies. YAC-FISH mapping demonstrated that the evolutionary rearrangements are more complex than those suggested by classical chromosome banding, involving breakpoints on HSA 3p24, 3p14, 3q13, and 3q21. Two pericentric inversions are required to derive the Borneo PPY 2 from the ancestral HSA 3. One pericentric inversion and one transposition are needed for the Sumatra PPY 2. In addition, several megabases of DNA from the pericentromeric region of HSA 3 are found at the short-arm telomere of both Borneo and Sumatra PPY 2.

Program Nr: 2018 from the 1999 ASHG Annual Meeting

Identification of balanced translocation t(5;9)(q15;p22.3) in amniocytes. *J.C. Shin, E.J. Baik, D.Y. Jung, Y. Lee, K.O. Kim, S.C. Kim, S.P. Kim.* Dept OB/GYN, Catholic Univ. of Korea, Medical Col, Seoul, Korea.

Standard cytogenetic analysis of amniocytes obtained at 19 weeks in gestation from a 36-year old pregnant woman revealed the balanced reciprocal translocation of chromosome 5 and 9. The G-banded karyotype was interpreted as 46,XY,t(5;9)(q15;p22.3). We performed chromosome analysis using GTG-banding from the parental peripheral blood. The parental chromosome analysis showed that the father carried the balanced translocation 46,XY,t(5;9)(q15;p22.3). Parental clinical features were normal. To clarify these results, FISH study were performed using whole chromosome painting probes 5 and 9. These study conformed the cytogenetic results. To our knowlege, this is the first report of 5q;9p balanced translocation in the prenatal diagnosis.

Program Nr: 2019 from the 1999 ASHG Annual Meeting

Spectral Karyotyping for marker chromosomes. *K. Siriwardena[1], R. Babul[1], D. Chitayat[1], C. Cytrynbaum[1], L. Dupuis[1], B.S. Emanuel[2], S. Kennedy[1], H. Kurahashi[2], G. Nie[1], A. Teebi[1], R. Weksberg[1], E. Winsor[3], I. Teshima[1]*. 1) Clin Genetics and Pediatric Lab Medicine, Hosp for Sick Children, Toronto,Canada; 2) Children's Hosp of Philadelphia, PA,USA; 3) Toronto Hosp,Canada.

Spectral karyotyping (SKY) can identify marker chromosomes not identifiable by G-banding. In a series of 17 cases, only eight were identified by G-banding and known clinical correlations. These were i(9p) and inv dup(15) [1 case each]; i(12p), i(18p) and bisatellited (22)[2 cases each]. However, nine cases (mostly mosaic) required SKY identification with followup FISH (locus specific /paints). These were two each of mar(1), mar(8) and mar(22), and one each of acentric mar (from 12pter), mar(15) and mar(16). Phenotypes of these markers identified by SKY compared to known reported abnormalities allows for genotype /phenotype correlations. Cases 1 and 2, both with markers originating from chromosome 1, presented with poor growth. Neither had striking facial dysmorphic features unlike the mosaic trisomy 1 case reported. Case 3 and 4 both with mar(8) have distinct phenotypes that share features and malformations seen in full mosaic trisomy 8. These include developmental delay, abnormal eye findings, structural abnormalities of the brain, scaphocephaly, cleft plate and external genitourinary abnormalities. Case 5 with mar(12) has global developmental delay as well as coarse facies and other dysmorphic features seen in Pallister-Killian syndrome (PKS). Craniofacial features included bifrontal sparseness of hair, abnormally placed prominent ears and mild hypertelorism. In addition, there were upslanting palpebral fissures with epicanthic folds, a flattened nasal bridge, a long philtrum with a prominent lower lip and a high-arched palate also seen in PKS. Case 6 with a nonsatellited marker derived from 15 was referred for poor growth and developmental delay. Case 7 was investigated for possible velocardiofacial syndrome but was shown to have a mar(16). Case 8 included a parent and infant with different markers derived from 22. Case 9 had a mar(22) that included the 22q11.2 region (D22S427, D22S36, TUPLE1, cHKAD26, HCF2, D22S801). Phenotypes and analyses of these cases will be discussed further.

Program Nr: 2020 from the 1999 ASHG Annual Meeting

Correlation between numerical and structural chromosome damage in sperm from 13 healthy men using multicolor FISH. *E. Sloter[1,2], J. Nath[2], A.J. Wyrobek[1].* 1) Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, CA; 2) Genetics and Developmental Biology Program, West Virginia University, Morgantown, WV.

Numerical and structural chromosomal damage transmitted via sperm have been associated with birth defects and genetic diseases. Our lab has developed and validated a multicolor FISH strategy for human sperm to simultaneously detect premeiotic and meiotic chromosomal abnormalities (numerical and structural) as well as postmeiotic structural chromosomal abnormalities using DNA probes for three target regions on chromosome 1. This includes the ability to detect (a) disomy 1 and diploidy, (b) partial duplications or deletions of 1p which are the consequence of premeiotic or meiotic rearrangements and (c) chromosome breaks within the 1cen-1q12 region which are presumably postmeiotic breakage events. We have analyzed ~100,000 total sperm from 13 healthy non-smoking men with no known exposure to genotoxic agents. A positive correlation ($P<0.01$) was found between the number of sperm with numerical abnormalities and the number of sperm with premeiotic or meiotic structural abnormalities. No association was found between the frequency of postmeiotic structural chromosomal defects and the frequency of premeiotic or meiotic abnormalities (numerical or structural). This finding suggests independent causal mechanism(s) for pre- and postmeiotic chromosomal damage in sperm. In addition, chromosomal breakage within the 1cen-1q12 region appeared to be non-randomly distributed. Multicolor FISH analysis of the centromere and adjacent heterochromatin of chromosome 1 revealed that >50% of the breaks within the 1cen-1q12 region occurred at or near the border between these two regions in 8 of the 13 men. There also appeared to be a non-random distribution of breaks within the large heterochromatic region. This sperm FISH methodology has promising clinical and toxicological applications for evaluating the etiology and risk factors associated with chromosomal aberrations in human sperm. [Work performed under the auspices of the US DOE by LLNL, contract W-7405-ENG-48 and funding from NIEHS Superfund P4ZES04705 and WVU].

Program Nr: 2021 from the 1999 ASHG Annual Meeting

Cytogenetic damage in human hematopoietic CD34+ progenitor cells. *M.T. Smith, M. Jeng, L. Zhang, Y. Wang, W. Guo, P. Duramad, G. Hofstadler, N.T. Holland.* Division of Environmental Health Sciences, School of Public Health, University of California, Berkeley CA 94720, USA.

Progenitor or stem cells are the likely target in the development of leukemias and other bone marrow (BM) disorders. Cytogenetic damage in these cells was assessed by two methods, micronucleus(MN) assay and fluorescent in situ hybridization(FISH). Micronuclei are easily measured under fluorescent microscopy. They consist of small amounts of DNA that arise in the cytoplasm when chromatid/chromosomal fragments or whole chromosomes are not incorporated into daughter nuclei during mitosis. We have used the MN assay to assess the baseline level of cytogenetic damage in progenitor cells enriched for CD34 antigen from human BM and umbilical cord blood(CB). To our knowledge no previous studies have used the MN assay in progenitor cells. Using a two-step immunomagnetic bead separation system (Miltenyi Biotech, Inc, CA), BM and CB progenitor cells were enriched for CD34 antigen. The purity of CD34+ in the enriched population was verified by FACScan analysis. The BM and CB progenitor cells were cultured for 72 hours in a media with an optimized combination of cytokines. MN frequency in CD34-enriched CB and BM was higher than that in CD34-negative cells. A lower MN frequency was noted in CB samples when compared to BM progenitor cells and peripheral lymphocytes, which is consistent with previous data showing that MN frequency is age-related. FISH analysis was performed in CD34-enriched cells, CD34-negative cells and granulocytes, using simultaneous hybridization with chromosome-specific probes for chromosomes 7 and 8. Monosomy 7 and trisomy 8 are commonly observed in leukemia. These changes have also been detected in the peripheral blood of workers exposed to benzene, established leukemogen. Like MN, significantly higher background levels of trisomy 7 and 8 were observed in CD34-enriched cells. Background levels of monosomy 7 and 8, however, were higher in CD34-negative cells for unclear reasons. Further studies with chemical exposures are underway to evaluate differential sensitivity of progenitor cells to in vitro cytogenetic damage.

Program Nr: 2022 from the 1999 ASHG Annual Meeting

Prenatal diagnosis and FISH characterization of an inherited ring (19) showing telomeric fusion and ring opening. *M.D. Speevak, G. Chow, C. Smart, J. Cardoso, S.A. Farrell.* Division of Genetics, Department of Laboratory Medicine, The Credit Valley Hospital, Mississauga, ON, Canada.

An amniotic fluid specimen from a 37-year-old phenotypically normal woman showed the presence of a single normal chromosome 19 and a ring (19) chromosome in cells of all colonies examined. Parental studies demonstrated that the father's chromosomes were normal. However, the mother was found to be a mosaic carrier of the ring (19) (4/100 cells examined). Following genetic counselling, the pregnancy was terminated. External examination of the male fetus revealed no obvious phenotypic abnormalities.

Fetal chromosome studies confirmed ring (19) in 19/20 cells examined. Dual colour FISH, using probes specific for the short and long arm subtelomeric regions of chromosome 19, was performed on cultured fetal cells. Hybridization signals were observed for each probe, indicating fusion of the chromosome 19 arms was distal to the probe loci. Our findings suggest that ring formation in this family arose from telomeric fusion rather than terminal deletion and joining of the chromosome arms. In the case of the fetus, mosaicism was likely due to *in vitro* opening of the ring (19), since a single normal male cell was detected in an otherwise abnormal colony of amniotic fluid cells. Perusal of the literature revealed no consistent correlation between cytogenetic data and the phenotype of individuals with ring (19). The counselling issues will be discussed.

Program Nr: 2023 from the 1999 ASHG Annual Meeting

Molecular cytogenetic characterization of the chromosomal region 2q37 in patients suffering from brachydactyly E including probes for the putative candidate gene LOBO1. *H. Starke[1], A. Rump[2], J. Seidel[3], V. Beensen[1], M. Stumm[4], J. Wirth[5], A. Heller[1], U. Claussen[1], T. Liehr[1]*. 1) Institute of Human Genetics and Anthropology, Jena, Germany; 2) Institute of Molecular Biotechnology e.V., Jena, Germany; 3) Childrens Hospital, Jena, Germany; 4) Institute of Human Genetics, Magdeburg, Germany; 5) Max-Planck-Institute for Molecular Genetics, Berlin-Dahlem, Germany.

We have identified the 3' portion of a large gene located on 2q37 (around STS marker WI8964), which leads to abnormal bone development in transgenetic mice. Given this murine phenotype, the human gene, which we call LOBO1, might be a candidate for brachydactyly E (Rump et al, 1999, Med. Genetik 11, 116). Brachydactyly E, the shortening of the metacarpals and phalanges of hands and/or feet, combined with mental retardation is suspected to be caused in some cases by small deletions in the subtelomeric region in 2q37, detectable only by molecular cytogenetics (Mc Kusick No. 600430). Here we report on 5 clinically confirmed cases suffering from brachydactyly-mental retardation syndrome. The following YAC clones have been used as probes to look for microdeletions: y780a10, y695g12, y770f05, y210e14, y854c09, y627d09, y963g7 and y764g8; additionally, a 3' derived LOBO1 cosmid probe has been used. In case 1 a micro-deletion in 2q37 could be detected, using the YAC y210e14 as probe. The deletion appeared in connection with a structural chromosome aberration described as 46,XY,add(2)(q37). After microdissection and reverse painting this translocation could be described as .rev ish der(2)t(2;acro)(q37;p11.2). As no deletion could be found with the 3' derived LOBO1 cosmid probe, further efforts will be done to clone the lacking parts of the human LOBO1 gene and complementary FISH studies with this new probes will be done. Moreover, to clarify if the LOBO1 gene is a candidate gene for brachydactyly E, mutation screening within the LOBO1 gene will be performed in case1. Acknowledgments: This work was supported by the Madeleine Buehler-Kinderkrebs-Stiftung and a fellowship to A. Heller and H. Starke by the Herbert Quandt Stiftung der VARTA AG.

Program Nr: 2024 from the 1999 ASHG Annual Meeting

Molecular Cytogenetic Analysis of a rec(7)dup(7q)inv(7)(p22q31.3)pat. *G. Stetten, K.M. Stone, J.M. Coddett, E.D. Gurewitsch, C.B. Cargile, K.J. Blakemore, B.K. Goodman.* Johns Hopkins University School of Medicine, Baltimore, MD.

Duplication and associated deletion within a single chromosome provides a unique opportunity to observe the consequences of gain and loss of specific genetic material. Current technology allows us to define the boundaries of these duplications and deletions with far greater resolution than previously possible. This is critical in order to facilitate direct karyotype-phenotype correlations. We have used a series of locus-specific and subtelomeric FISH probes to define a recombinant chromosome 7, involving a large duplication (7q31.3-qter) and a very small terminal deletion (7p22). The chromosome was detected at amniocentesis following an ultrasound study that revealed hemivertebrae and a two-vessel umbilical cord. The abnormality arose during meiotic recombination of a paternal pericentric inversion chromosome, inv(7)(p22q31.3).

Following G-band analysis, six DNA probes were mapped by standard FISH techniques to refine the short and long arm breakpoints. A probe mapping to 7p22 (containing D7S589) (Oncor) hybridized to the abnormal short arm in the rec(7), but a 7p telomere-specific probe (Vysis) was deleted from this chromosome. The long arm breakpoint was narrowed to an interval between markers D7S490 and D7S686, less than 0.1Mb, using the father's inv(7) (probes kindly provided by S. Scherer).

Prenatal and postnatal findings included skeletal abnormalities, dilated cerebral ventricles, microretrognathia and a short neck. The clinical phenotype appeared to result primarily from duplication of the long arm segment. Comparison of these findings to previously reported cases of terminal duplication 7q indicated good agreement with a consensus phenotype.

Program Nr: 2025 from the 1999 ASHG Annual Meeting

Subtle overlapping deletions in the terminal region of chromosome 6q24.2-q26: Three cases studied using FISH.

S. Sukumar[1], S. Wang[1], C.M. Vanchiere[2], K. England[3], R. Fick[4], B. Pagon[5], K.S. Reddy[1]. 1) Cytogenetics, Quest Diagnostics Inc., San Juan Capist, CA; 2) Childrens Clinic of Southwest Louisiana, LA; 3) Casa Blanca Clinic, Mesa, AZ; 4) Mary Bridge Childrens Health Center, Tacoma, WA; 5) Childrens Hospital and Regional Medical Center, Seattle, WA.

Interstitial deletions in the terminal region of chromosome 6 are rare. Three new cases with subtle interstitial deletions in the q24-q26 region of the long arm of chromosome 6 are described in this study. The karyotypes were analyzed at 550 band level. Patient-1 is a nine-month old boy with an interstitial deletion, del(6)(q24.2q25.1), developmental delay, low birth weight, hypotonia, heart murmur, respiratory distress, craniofacial and genital anomalies. This is the first report of a case with deletion, del(6)(q24.2q25.1). Patient-2 is a 17-year old male with an interstitial deletion, del(6)(q25.1q25.3), developmental delay, short stature, mental retardation, autism, head, face, chest, hand and feet anomalies and a history of seizures. For the first time autism has been described as a clinical feature in 6q deletions. Patient-3 is newborn baby boy with a de novo interstitial deletion, del(6)(q25.1q26), developmental delay, anomalies of the brain, genital organs, limbs and feet. This is the first report of a case with deletion, del(6)(q25.1q26). In all the patients, fluorescence in situ hybridization (FISH) using chromosome 6 painting probe ruled out a possible insertion. The ESR (6q25.1) and TBP (6q27) probes are used to confirm the breakpoints. Since TBP signal is present in all cases, it confirmed an interstitial deletion proximal to this probe. Patient-1 has a deletion of the ESR locus; Patient-2 and 3 have a signal for the ESR locus. Therefore the deletion in both these patients are distal to Patient-1 between ESR and TBP loci. FISH validated the deletion breakpoints assessed by conventional cytogenetics.

Program Nr: 2026 from the 1999 ASHG Annual Meeting

An analphoid marker chromosome shown to be an inverted duplication 8q23qter with a neocentromere. *V. Sulcova[1], K.S. Reddy[1], S. Schwartz[2], J. Noble[3], H. Lin[3]*. 1) Cytogenetic, Quest Diagnostics Inc, San Juan Capist, CA; 2) Department of Genetics and Center for Human Genetics, Case Western Reserve University School of Medicine and University Hospitals of Cleveland, Cleveland, OH; 3) Department of Medical Genetics, Harbor UCLA, Torrance, CA.

We report a new marker chromosome that was acentric but stable through cell division in a 15-year-old with a history of developmental delay, mental retardation and behavioral problems. The patient was hirsuit with polycystic ovaries and spasticity of the lower extremities. She was recently diagnosed with myasthenia gravis, presumed to be an ocular variant. G-banded chromosome analysis revealed a marker chromosome in 75% of metaphases studied. The marker was C-band negative. By FISH alpha-satellite sequences were not detected on the marker. Subtelomeric probe for 8q hybridized to the two ends of the marker and the c-myc probe that maps to 8q24 also gave two signals. The marker was shown to be entirely chromosome 8 material by using coatasome 8 probe, which painted the marker uniformly end to end. The G-bands gave the appearance of mirror image duplication. This was confirmed by the pattern of the two telomeric, subtelomeric and c-myc probe signals. The marker was concluded to be an inverted duplication 8q23qter, and the karyotype was 46,XX,der(8)(qter®q23::q23®[neocen]®qter). Both parents had normal karyotypes. The marker was found in 84%lymphoblastoid culture cells. Centomeric proteins CENPC, CENPE and not CENPB were present at a position defining a neo-centromere. This 8q marker that appears analphoid by FISH was remarkably stable in mitosis and was shown to have the known critical centromeric proteins at its neocentromere.

Program Nr: 2027 from the 1999 ASHG Annual Meeting

Should patients with a triploid pregnancy be offered prenatal diagnosis in subsequent pregnancies? *J.C. Sullivan, G. Cohn, T. Marini, R. Naeem.* Baystate Medical Ctr, Springfield, MA. Departments of Pathology and Obstetrics and Gynecology.

Triploidy is estimated to occur in approximately 2% of all conceptuses. Recurrence risk for triploidy or aneuploidy in subsequent pregnancies has been discussed in a handful of papers and abstracts (Inati et al., Uchida and Freeman, and Warburton et al.). It has been suggested that recurrence of triploidy may simply be due to chance given the small number of cases. Yet, controversy remains as to whether the occurrence of a triploid pregnancy confers an increased risk for another triploid and/or aneuploid. This question is of practical importance in the clinical setting where couples seek options and recommendations for prenatal diagnosis in pregnancies following a triploid conception. During a five and a half year period our cytogenetics laboratory identified 121 consecutive cases of triploidy in products of conception, stillbirths, and intrauterine fetal demises from 118 women. Three women (2.5%) had consecutive triploid pregnancies. Five of 118 (4.2%) women had a previous or subsequent pregnancy with aneuploidy (3 trisomies and 2 tetraploids). Three of the aneuploidies were trisomies, only one of which occurred in a patient with advanced maternal age. Although these numbers are small and may indeed be due to chance, recurrence risk for triploidy remains an ongoing clinical dilemma. Larger studies or pooled data from several surveys are necessary to lay the controversy to rest and provide clinical guidelines for counseling patients following a triploid conceptus.

Program Nr: 2028 from the 1999 ASHG Annual Meeting

Identification of a cryptic reciprocal translocation t(5;10) by FISH resulting in 5p microdeletion in two subsequent pregnancies. *F.E. Tahmaz[1], M. Freidine[2], M.M McCorquodale[2], B.K. Burton[3]*. 1) Dept Pediatrics, Div Genetics, Univ Illinois at Chicago, Chicago, IL; 2) Cytogenetics Laboratory, Michael Reese Hospital Medical Center, Chicago, IL; 3) Division of Genetics, Children's Memorial Hospital, Northwestern University, Chicago, IL.

Deletion 5p or Cri du Chat syndrome is characterized by partial deletion of short arm of chromosome 5. It has been reported that approximately 85% of the cases result from sporadic de novo deletions, whereas 15% are secondary to unequal segregation of a parental translocation. We report here a case of a 38 year-old female with two miscarriages and a child with Cri du Chat Syndrome. Her first pregnancy ended in a miscarriage at the 12^{th} week of gestation. Subsequently she had a child with the phenotypic features of Cri du Chat syndrome including postnatal growth retardation, microcephaly, hypertelorism, facial asymmetry, low-set dysplastic ears, VSD, unilateral simian crease, developmental delays, seizures and the typical high pitched cry. FISH analysis using the probe D5S23, specific for the 5p15.2 Cri du Chat critical region, revealed the presence of 5p microdeletion. In her third pregnancy, which resulted in a miscarriage at the 14^{th} week, prenatal FISH analysis of CVS again showed absence of one of the 5p critical regions. High resolution chromosome analysis revealed normal karyotypes in both parents. In addition, FISH analysis for the 5p critical region showed two signals in both parents. However, subsequent FISH studies in the mother using whole chromosome paints for chromosomes 5 and 10 revealed a cryptic translocation between distal portions of 5p and 10p involving the bands p14.2 and p12.2 [46, XX, t (5;10) (p14.2;p12.2)]. While most cases of Cri Du Chat Syndrome are caused by de novo deletions, this case demonstrates the importance of performing FISH studies in the parents of 5p microdeletion cases in order to rule out the presence of small cryptic translocations.

Program Nr: 2029 from the 1999 ASHG Annual Meeting

Stability of transferred human chromosomes in human and mouse cells. *S. Takehara[1], T. shinohara[1], K. Yamauchi[1], K. Tomizuka[2], A. Ohguma[2], I. Ishida[2], M. Oshimura[1].* 1) Dept Mol Cell Genet, Tottori Univ Sch Life Sciences, Yonago, Tottori, Japan; 2) Pharmaceutical Research Laboratory, KIRIN Brewery Co., Ltd., Japan.

Introduction of human chromosomes or fragments into variable cells via microcell- mediated chromosome transfer (MMCT) is an useful technique for mapping, cloning particular genes and functional studies. The MMCT made it possible to (1) transfer intact or megabase regions of chromosomal fragments, (2) study epigenetic modifications such as genomic imprinting. Thus, it is important for the transferred chromosome to be maintained in host cells. To examine the stability of transferred human chromosome, chromosomes 2, 11, 14, 21 and/or these fragments were transferred into the mouse fibrosarcoma cell line (A9), the mouse embryonic stem cell line (ES), the chicken pre-B lymphoid cell line (DT40), the human cervical tumor cell line (Hela), the human fibrosarcoma cell line (HT1080), and then cultured with or without selection. The intact or fragments of human chromosome 2, 11, 21 were lost at ~100 PDL, but chromosome 14 or the fragment were stably maintained at least at 100 PDL in all the cell lines examined. We also examined the retention of the human chromosome 14 fragment (~10Mb) in trans-chromosomal mice (F1 and F2), which were produced from ES cell line containing a human chromosome 14 fragment. Fluorescence in situ hybridization (FISH) analyses revealed that the single human chromosome 14 fragment was stably maintained at least in brain and liver tissues. FISH analyses using a human chromosome 14 centromere specific probe, mouse major and minor satellite specific probes confirmed that the human chromosome 14 fragment consisted of human centromere. Thus, some human chromosomes are stable in variable cell types, including tumor cells and mouse ES cells, and that some chromosome fragments can be also stably transmitted through germline. In future, the human chromosome 14 fragment may be useful for developing as a vector for gene delivery into mouse and human.

Program Nr: 2030 from the 1999 ASHG Annual Meeting

Numerical Chromosome Aberrations in Normal and Patient Cells Using PNA Probes by FISH. *K.L. Taneja[1], C. Whitney[2], E.A. Chavez[3], J. Coull[1], P. Newburger[2], P.L. Lansdorp[3].* 1) Boston Probes, Inc, Bedford, MA; 2) Department of Pediatrics, U. Mass. Med. School, Worcester, MA; 3) Terry Fox Laboratory, BC Cancer Research Center, Vancouver, Canada.

We have developed multicolor fluorescence in situ hybridization to detect and localize DNA repeat sequences within single cells. Telomeric and centromeric repeat sequences were detected in chromosomes of human metaphase spreads using PNA probes labeled with different fluorochromes. Using specific PNA probes for chromosome X, Y and 1, we could easily distinguish chromosomal aneuploidy and polyploidy by four color analysis in patient cells with known autosomal and sex chromosomal abnormalities. With specific PNA probes for chromosomes X, Y, 1, 2, 6, 10, 17 and 18, we performed a FISH analysis on different HL60 cell lines. Trisomy or tetrasomy of chromosome 18 was present in all cell lines, whereas tetrasomy of chromosomes 2, 6, 10 and 17 with two copies of X chromosome, as well as an extra copy of dicentric chromosome 1 was observed with two cell lines. Three cell lines showed one copy of X chromosome and two out of three cell lines showed trisomy of chromosome 6. The Y chromosome was not detected in any of the cell lines. These results demonstrated the usefulness of chromosome specific PNA probes in the analysis of numerical chromosomal abnormalities and highlight the difficulty in comparing results of all biological experiments with HL60 cells between laboratories.

Program Nr: 2031 from the 1999 ASHG Annual Meeting

Mosaicism for Angelman syndrome. *M. Tekin[1], C. Jackson-Cook[1], A. Buller[2], A. Ferreira-Gonzalez[2], A. Pandya[1], J. Bodurtha[1]*. 1) Dept of Human Genetics, Virginia Commonwealth Univ, Richmond, VA; 2) Dept of Pathology, Virginia Commonwealth Univ, Richmond, VA.

We present a 28-month-old Caucasian female with developmental delay, severe speech impairment,inappropriate happy demeanor, a wide-based gait, frequent ear infections with mild hearing loss, and distinctive facial features including deep-set eyes, a wide mouth and widely-spaced teeth. A FISH chromosome analysis, using probes D15Z1, D15S10, and SNRPN, revealed mosaicism for the presence of a structurally abnormal chromosome 15 that was characterized by a duplication of the centromeric region and loss of the proximal long arm [dic(15)(pter ® q11.1::p11.2 ®q11.1::q13® qter)]. The aberrant chromosome was present in 40% of peripheral blood lymphocytes and was inferred from interphase FISH studies to be present in 30% of buccal mucosa cells. Parental chromosome studies were within normal limits, indicating that the aberrant chromosome formed as a de novo event (as expected from the mosaicism). Methylation studies using PCR with sodium bisulfite treated DNA revealed a normal biparental methylation pattern. Although the patient's facial, behavioral, and neurological findings were suggestive of Angelman syndrome (AS), developmental delay seemed to be milder than that observed classically in this syndrome. She also currently does not have microcephaly and seizures, which have been noted in most patients with AS. Although rare, mosaicism has been reported for Prader-Willi syndrome (PWS). The clinical presentation of these cases ranged from classical PWS phenotype to atypical, but PWS-like features. To the best of our knowledge, this is the first case of Angelman syndrome with a FISH-detectable deletion in a mosaic pattern. We recommend FISH studies for the detection of mosaicism in patients with Angelman syndrome clinical findings, even if methylation analyses show a normal pattern.

Program Nr: 2032 from the 1999 ASHG Annual Meeting

A GIRL WITH CORNELIA DE LANGE SYNDROME PRESENTING A DE NOVO 1q41->qter TRISOMY REVEALED BY SPECTRAL KARYOTYPING (SKY). *L. Telvi*[1], *R. ION*[1], *G. PONSOT*[2]. 1) LABORATOIRE DE CYTOGENETIQUE, HOPITAL ST VINCENT DE PAUL, PARIS, FRANCE; 2) SERVICE DE NEUROPEDIATRIE, HOPITAL ST VINCENT DE PAUL, PARIS, FRANCE.

We describe a girl presenting Cornelia de Lange syndrome with characteristic face, short stature (-2SD), large thorax, hypoplastic thenar and feeding difficulties. The blood karyotype after RHG and high resolution banding showed the 46,XX,5q+; pattern, with an unknown trisomic material at the end of the long arm of the chromosome 5. The whole paint of chromosomes 5 and 3 labelled to digoxigenin (ONCOR) showed that the extra piece do not belong to these chromosomes. Spectral karyotyping (SKY) revealed that the extra piece on chromosome 5 originated from chromosome 1. This was confirmed by chromosome painting. The karyotype of the child is thus 46,XX,der(5)t(1;5)(q41;q35). The patient has partial trisomy of chromosome 1. These findings emphasize the importance of using advanced techniques as the Multicolor karyotype to characterize extra material in chromosomes. Recent attention has focused on 3q as the localisation of the Cornelia de Lange gene. Ireland et al. suggested that the critical region for the Cornelia de Lange syndrome is between 3q25.1 and 3q26.3. The reported case raises the question of the involvement of the 1q41->qter trisomy and the implicated breakpoints in the pathogenesis of this syndrome.

Program Nr: 2033 from the 1999 ASHG Annual Meeting

Low Grade Mosaicism for a Derivative Y with Partial 5q Trisomy in a Small Male. *J.H. Tepperberg[1], R.E. Schnur[2], P.R. Papenhasuen[2], I. Gadi[1], P.N. Mowrey[1], L. Howard[2]*. 1) Dept Cytogenetics, Laboratory Corp of America, Res Triangle Pk, NC; 2) Div Genetics, Cooper Hosp/UMC Benson St, 3rd Fl Camden, NJ.

Duplications of chromosome 5 distal long arm (q33->35) are associated with severe psychomotor retardation, intrauterine growth retardation, brachydactyly, microcephaly, and facial abnormalities. The patient had mild facial abnormalites. External genetalia appeared normal although the scrotum and testicals were small. LH and FSH levels were normal. Kidneys were normal. When last seen at 2 and 1/2 years he demonstrated normal cognitive and motor development.Cytogenetic analysis of a 1 year old male referred for developmental delay showed a 5,X[48]/46,X,der(Y) t(Y;5)(p11.3;q33)[2]. The identification of the abnormal Y chromosome was confirmed by FISH, using a Y pericentromeric DNA probe. A multiprobe FISH device was used to show that the additional chromosome material was derive from chromosome 5. The 5q33->5qter origin was based on the G-banding and absence of a chromosome 5 unique sequence short arm FISH probe. PCR for the testis determining region (SRY) showed the presence of SRY. Additional FISH on a buccal smear is being evaluated to determine the percentage of mosaicism of the derivative Y in another tissue. Paternal chromosome analysis showed a normal 46,XY karyotype. Thus, this patient is partially trisomic for 5q and nullosomic for distal chromosome Y, in those containing the derivative Y chromosome.

Program Nr: 2034 from the 1999 ASHG Annual Meeting

Clinical application of PRINS as an alternative to FISH in the diagnosis of Prader Willi/Angelman and DiGeorge syndromes. *A.T. Tharapel, J.S. Kadandale.* Clinical and Molecular Cytogenetics Laboratory, Department of Pediatrics, University of Tennessee, Memphis, TN.

Diagnoses of Prader Willi/Angelman (PW/AS) and DiGeorge syndromes (DGCR) are facilitated by FISH with DNA probes spanning the critical region of the deletion. But a newly-developed methodology, primed in situ labeling (PRINS), can be adapted to the diagnosis of microdeletions. To demonstrate the utility of PRINS in the detection of microdeletions, we studied 3 patients each with PW and DGCR syndromes. Deletions in these patients were previously confirmed by FISH. Two normal individuals served as controls for each experiment. Oligonucleotide primers for SNRPN and GABRB3 for PW syndrome, and D22S1638 and D22S1648 for DGCR syndrome were synthesized locally. The reactions were performed separately with primers for the respective loci. Annealing and extension were done on chromosome preparations in the presence of labeled nucleotides and Taq DNA polymerase. For each patient, 50 metaphase spreads were analyzed under the fluorescence microscope. The 3 patients with PW syndrome showed absence of labeling (for SNRPN and GABRB3) on one chromosome 15 representing the deletion. Similarly, 3 patients with DGCR syndrome showed deletion for the D22S1638 and D22S1648 loci on one of the chromosomes 22. A normal pattern for all loci was obtained in controls. In view of its sensitivity, specificity and cost effectiveness, PRINS can thus serve as an alternative to FISH in the diagnosis of microdeletion syndromes.

Program Nr: 2035 from the 1999 ASHG Annual Meeting

Trisomy 10 mosaicism detected prenatally and confirmed in fetal tissues by interphase FISH. *D. Thibodeau, J. Bergoffen, B. Saxon, D. Sipos, K. Nguyen, S. Owen, L. Bros, X. Li.* Genetics Dept, Kaiser Permanente Medical Group, San Jose, CA.

Trisomy 10 mosaicism is a rare chromosome anomaly. Significant features in five liveborn cases include failure to thrive, dysmorphic facies, cardiopathy, and early death. We present a case of trisomy 10 mosaicism detected prenatally by amniocentesis and confirmed in various fetal tissues using interphase fluorescence in situ hybridization (FISH).

Genetic amniocentesis for maternal age 35 was performed at 13.7 weeks gestation by ultrasound dating. Two colonies from one in situ culture showed trisomy 10. The remaining 19 colonies in five cultures revealed a normal karyotype (46,XX). From a repeat amniocentesis, standard cytogenetic analysis of 25 colonies revealed a normal female karyotype. Analysis of a direct preparation of the amniotic fluid by interphase FISH using an alpha satellite DNA probe specific for chromosome 10 (Vysis, Inc.) showed trisomy 10 in 12% of cells. Fetal anatomy and interval growth were normal by serial ultrasound.

The pregnancy was terminated by D&E at 19.8 weeks gestation. No trisomy 10 cells were found in 75 metaphases analyzed from cultures (50 from kidney, 25 from heart). Interphase FISH analysis of these cultured cells revealed 12% and 0% trisomy 10 cells, respectively. Interphase FISH analysis of the direct preparations (touch prep) of 14 different fetal tissues showed the following proportions of trisomy 10 cells: kidney (23%), heart (27%), lung (12%), ovary (10%), fascia lata (9%), amnion (9%), umbilical cord (7%), muscle (6%), skin (1%), villi (1%), liver (0%), intestine (0%), rib (0%), and head soft tissues (0%). Pathology evaluation of highly fragmented products of conception failed to reveal any gross or microscopic abnormality.

Our findings indicate that the trisomy 10 cells might have been selectively lost due to a growth disadvantage in vitro. We highly recommend prenatal and postnatal interphase FISH on direct tissues for confirmation of fetal trisomy 10 or other mosaicism.

Program Nr: 2036 from the 1999 ASHG Annual Meeting

Trisomy 21 mosaicism in monozygotic twins discordant for Down syndrome. *H. Thiele[1], P. Wiegand[2], C. Baldermann[1], H. Schmidt[3], W. Knopp[4], I. Hansmann[1], U. Hehr[1].* 1) Humangenetik, Martin-Luther-Universität, Halle, Germany; 2) Rechtsmedizin, Martin-Luther-Universität, Halle, Germany; 3) Pathologie, Martin-Luther-Universität, Halle, Germany; 4) Kinderklinik, Weissenfels, Germany.

Monozygotic twins typically are equipped with identical sets of chromosomes and hence expected to present with similar phenotypic features. The few reported instances of discordance for monogenic conditions or chromosomal aberrations were attributed to incomplete penetrance, a wide phenotypic spectrum of the observed syndrome or to mosaicism. Here we present the clinical, cytogenetic and molecular data of monozygotic twins discordant for Down syndrome. At the age of 4 months the first twin presented with short stature, hypotonia and typical craniofacial features of Down syndrome, while here twin sister did not show any abnormalities. Repeated chromosome analysis from lymphocytes revealed for both twins an identical diagnosis: trisomy 21 was found in appr. 4-5% of all analyzed cells. This observation confirmed trisomy 21 mosaicism in both girls and suggested them to be monozygotic. By chromosome analysis from skin fibroblasts the phenotypically normal twin was found to have a normal karyotype in all analyzed cells while trisomy 21 was found in 14 of 21 cells of her affected twin sister. Reexamination of both girls at the age of 17 years confirmed the previously established clinical diagnosis. The impaired physical performance of the twin with Down syndrome was further restricted because of a complex cardiac malformation, she was unable to communicate by intelligible speech. In contrast, her twin sister presented both physically and mentally as a normal teenager. In addition, a psychological test at the age of 3.5 years had confirmed her mental abilities to be within normal range. Monozygosity was established using 16 informative marker from 12 chromosomes (>99.9% probability). Reevaluation of the chromosomes from lymphocytes again documented trisomy 21 mosaicism in the affected twin (1/100 cells) and her normal sister (2/100 cells). These twin sisters once more impressively demonstrate the wide phenotypic spectrum observed in probands with weak trisomy 21 mosaicism in blood cells.

Program Nr: 2037 from the 1999 ASHG Annual Meeting

A cytogenetic and clinical evaluation of a de novo duplication 7(q36.1®qter). *K.J. Thompson, G.S. Sekhon, D. Wargowski, H. Singh.* Dept Genetics, Univ Wisconsin, Madison, WI.

We report a 4-year-old with multiple anomalies caused by a de novo duplication of the long arm of chromosome 7. The patient exhibited developmental delay, hydrocephalus, cleft lip and palate. High resolution chromosome analysis of 75 lymphocyte metaphases revealed: 67% of cells with 46,XX,dup(7)(pter®q36.3::q36.1®qter) and 33% with a normal female karyotype. Fluorescent in-situ hybridization approaches, including use of regional specific cosmid probes, were used to confirm the chromosome 7q origin of the duplicated material. The possible mechanisms of formation of the duplication will be discussed.

Program Nr: 2038 from the 1999 ASHG Annual Meeting

Ring 4 chromosome in two sibs with abnormal growth pattern and apparently normal parents. *D.J. Tomkins[1], R. Couch[2], J. Chernos[3,4], J. Gerritsen[3], D.R. McLeod[3]*. 1) Dept Medical Genetics, Univ Alberta and Cytogenetics, UA Hospital, Edmonton, AB, Canada; 2) Dept Pediatrics, Univ Alberta and UA Hosp; 3) Dept Medical Genetics, Univ Calgary and Alberta Children's Hosp, Calgary, AB; 4) Cytogenetics, Alberta Children's Hosp.

A large ring chromosome 4 was found in a girl with a profound growth abnormality. She was karyotyped at age 1 year 9 months because of small size since birth and a question of Turner syndrome. All cells but one from a peripheral blood sample had the karyotype: 46,XX,r(4)(p16q35). One open duplicated ring was found out of 30 cells. At two years of age, her growth was well below the 3rd %ile for weight and height, but her endocrine studies and developmental milestones were normal. The same pattern of growth was recognized in a daughter born three years after the proband. Chromosomal analysis revealed the same ring 4 chromosome in 25 out of 31 metaphases with monosomy 4 in 3, monosomy 4 plus a marker in 2 and a duplicated ring in 1 cell. No ring was found in 30 cells from peripheral blood specimens from either parent or in fibroblast cultures from the mother; the father declined a skin biopsy. Gonadal mosaicism in a parent remains the most likely explanation for the recurrence of the ring 4. A general ring syndrome of severe growth retardation without major anomalies has been attributed to loss of cells with imbalances due to instability of rings (Côté *et al*., 1981). Two cases of ring 4 in children with severe growth retardation and mild dysmorphism were shown by FISH to have an intact 4pter (Pezzolo *et al*., 1993; Calabrese *et al*., 1997). This suggested that the growth retardation was due to the general ring syndrome; however, the 4qter region was not studied. The ring 4 in the present study was investigated by FISH with subtelomeric probes for 4p and 4q. The 4p subtelomeric region was intact but there was no 4qter fluorescent signal on the ring chromosome indicating a deletion within 400 kb of the telomere. This suggests that there may be a critical region for normal growth in the 4q subtelomeric region. Further molecular analysis is underway to determine the extent of the deletion in the 4qter region.

Program Nr: 2039 from the 1999 ASHG Annual Meeting

Evaluation of the chromosomal contents of micronuclei (MN) using spectral karyotyping (SKY) and FISH. *N. Tszine, C. Jackson-Cook.* Dept. of Human Genetics, Medical College of Virginia of Virginia Commonwealth University, Richmond VA.

DNA loss by the process of micronucleation is associated with aging, cancer and environmental exposure. The primary aim of this study was to identify the chromosomal content of the DNA being extruded into MN. This was achieved using our novel application of SKY and FISH technologies. To our knowledge, this study is the first to evaluate MN using SKY. Cytochalasin B (CytB) treated lymphocyte cultures from 3 females (ages 28,42 and 72) were analyzed. SKY revealed that the majority of MN (89.8%, 82.9%, 97.3% in the 28,42 and 72 year-old (y.o.), respectively) had a uniform, single color, suggesting that they were comprised of DNA from a single chromosome. Overall, the X chromosome was seen most frequently. However, a significant difference was noted (confirmed using FISH with a whole chromosome paitning probe (wcp) for the X chromosome) in its frequency among individuals (46.1% in the 42 y.o., 12.5% in the 28 y.o., and just 5% in the 72 y.o.). SKY also allowed for the identification of MN that contained autosomes. In fact, all 22 autosomes were observed to be excluded into MN in the 28 y.o. individual. Overall, the confirmation studies (using FISH with wcp) were in agreement with the SKY assignments for 73.5% of the MN. Studies are ongoing to identify refinements in the SKY procedure that will result in a higher accuracy level. The subset of MN with confirmed chromosomal contents showed no correlation between micronucleus size and the size of the chromosome it contained. Sequential staining of the same MN, using a pancentromeric probe, revealed 78.7% to have one signal. The frequency of MN was also compared between CytB treated and untreated cultures from these females. In 2/3 cases a significant difference in MN frequencies was observed ($p<0.001$ for the 28 y.o. and $p=0.019$ for the 72 y.o.). Collectively, these study results support the hypothesis that MN induced by CytB contain DNA from a single, monocentric chromosome (probably due to chromosome loss). The observed variation in response to the CytB treatment raises the possibility of individual-specific susceptibility to exogenous agents that potentially could influence chromosome segregation.

Program Nr: 2040 from the 1999 ASHG Annual Meeting

DNA Demethylation, Heterochromatin Decondensation, and Generation of Pericentromeric Rearrangements in Chromosomes 1 and 16 in B Cell Lines from Patients with the ICF Syndrome.. *C.M. Tuck-Muller[1], A. Narayan[2], F. Tsien[2], D. Smeets[3], E. Fiala[4], O. Soon[4], M. Ehrlich[2].* 1) Dept Medical Genetics, Univ South Alabama, Mobile, AL; 2) Human Genetics Program/Hayward Genetics Center, Tulane Univ Medical School, New Orleans, LA; 3) Human Genetics, Univ Hospital Nijmegen, Nijmegen, The Netherlands; 4) American Health Foundation, Valhalla, NY.

One of the diagnostic features of the rare, recessive ICF syndrome (immunodeficiency, centromeric region instability, facial anomalies) is rearrangements involving the juxtacentromeric heterochromatic regions of chromosomes 1 or 16 (1qh and 16qh) in mitogen-stimulated lymphocytes. ICF leukocytes display abnormal hypomethylation of satellite 2 DNA which is primarily located in 1qh and 16qh. We show that in B cell lines from two ICF patients the 1qh and 16qh regions are frequently rearranged while satellite 2 DNA hypomethylation characteristic of in vivo-derived ICF cells is maintained. These rearrangements include multibranched chromosomes with 3-6 chromosome arms observed in up to 22% of the metaphases, whole-arm deletions, and pericentromeric translocations, as seen in mitogen-stimulated T cells from ICF patients. An even higher percentage of the cultured ICF B cells display decondensation in the normally highly condensed 1qh and 16qh regions. Telomere associations, not previously reported in ICF syndrome, were seen in up to 14% of cells. Fusions between 1qh or 16qh and telomeric regions of other chromosomes were also observed. We propose that ICF-specific DNA hypomethylation predisposes the heterochromatin of chromosomes 1 and 16 to decondensation, which in turn, leads to rearrangements targeted specifically to these hypomethylated regions. We also demonstrate by high performance liquid chromatography of digests of DNA from ICF autopsy tissue, as well as of ICF B cell lines, that the extent of overall hypomethylation of genomic DNA in ICF is much less than that of satellite 2 DNA. Therefore, ICF, the only human genetic disease known to involve Mendelian inheritance of a DNA methylation anomaly, has hypomethylation restricted to only a portion of the genome.

Program Nr: 2041 from the 1999 ASHG Annual Meeting

A hierarchical approach of chromosome analysis employing multiplex-FISH, multicolor bar codes, and locus specific hybridizations in pre- and postnatal diagnostic applications. *S. Uhrig[1], S. Schuffenhauer[2], C. Fauth[1], A. Wirtz[2], J. Kraus[1], C. Daumer-Haas[3], M. Cohen[4], C. Apacik[4], T. Cremer[1], J. Murken[2], M.R. Speicher[1]*. 1) LMU-Munich, Institute of Human Genetics, Munich, Germany; 2) Abteilung Medizinische Genetik der Kinderpoliklinik der Universitt Mnchen, Munich, Germany; 3) Prnatal-Medizin, Lachnerstr. 20, Munich, Germany; 4) Kinderzentrum Mnchen, Heiglhofstr. 63, Munich, Germany.

A hierarchical approach of chromosome analysis is presented. The first step in this approach is the application of multiplex-FISH (M-FISH) which allows to identify the twenty-four different human chromosomes in a metaphase spread based on different colors. This initial M-FISH experiment determines the appropriate subsequent step or steps to take. These steps can include a variety of different techniques, such as CGH, reverse painting, locus specific hybridization or multicolor-chromosome-specific-bar-codes. With this hierarchical approach we have already analyzed more than 150 cases. Our experiences with the first 120 cases will be published shortly (Uhrig et al. Am J Hum Genet, in press). Here we will focus on additional cases. Specifically, the potential of M-FISH to characterize the origin of euchromatin in supernumerary marker chromosomes occurring in only 10% of amniotic fluid cells was shown in two cases where the euchromatin was derived from chromosome 22 or 19. In addition, we show examples, in which M-FISH found abnormalities not visible by GTG-banding alone. One of these cases involved a mosaic deletion of chromosome 11q. However, M-FISH revealed that the "normal" cells had in fact a cryptic translocation t(11;13). In our ongoing study in which M-FISH is used to screen metaphase spreads defined as normal by G-Banding in patients with a phenotype highly suggestive for a chromosomal syndrome we can add two new cases in which M-FISH identified unbalanced translocations. Overall, in this patient group we identified rearrangements in about 10%. M-FISH and chromosome-specific-bar-codes improve cytogenetic analysis and therefore should contribute to a better phenotype-genotype correlation and improve genetic counseling.

Program Nr: 2042 from the 1999 ASHG Annual Meeting

Molecular mechanism and consequence of intrachromosomal triplication of proximal 15q. *P. Ungaro, S.L. Christian, J. Fantes, D.H. Ledbetter.* Dept. of Human Genetics, University of Chicago, Chicago Illinois.

Deficiencies of paternal or maternal contributions of chromosome 15q11-q13 due to deletion or uniparental disomy are known mechanisms for Prader-Willi syndrome (PWS) and Angelman syndrome (AS). In the present study we describe the molecular characterization of the 15q11-13 region in four patients with severe mental retardation and some features similar to PWS or AS. Fluorescence in situ hybridization (FISH) was performed using BACs or PACs identified with STSs for D15S18, *UBE3A*, D15S1019, D15S165 and D15S144. In all four patients three copies of 15q11-q13 were identified on one homolog. In three of them the triplicated region involved the most proximal breakpoint (BP1) and a distal breakpoint that lies between D15S1019 and D15S165. In all patients the triplication appears to have the same normal-inverted-normal orientation. Microsatellite analysis was performed to establish the parental origin of the triplication. Results show that in three cases the triplication is derived from a maternal unequal recombination event and in the fourth from a paternal one. To determine how the triplication affects the methylation status, exon alpha of the SNRPN gene was analyzed by M-PCR. The patient that received the triplicated allele from the father shows a paternal product three times more intense than the maternal one, while the others, who inherited the triplication from the mother, have a maternal product approximately three times more intense than the paternal one. In addition expression of the SNRPN gene was analyzed by northern blot and was found to be concordant with the inheritance of the triplication, i.e. SNRPN expression in the patient that inherited the triplication from the father is three times higher than normal controls and the patients with a maternal triplication. In conclusion, our data indicate that both the methylation pattern and SNRPN expression show a dose-dependent increase that correlates with the parental origin of the triplication. The mechanism involved in the origin of the triplication remains to be clarified, however the orientation (N-I-N) of the triplication in all our patients suggests a common mechanism of rearrangement.

Program Nr: 2043 from the 1999 ASHG Annual Meeting

Interphase FISH analysis of the PLP gene in Pelizaeus-Merzbacher disease. *G.H. Vance[1], O. Henegariu[2], V.C. Thurston[1], L. Sendi[1], S. Dlouhy[1], J.A. Trofatter[3], M.E. Hodes[1].* 1) Dept Med & Molec Genetics, Indiana Univ, Indianapolis, IN; 2) Dept of Genetics, Yale University, New Haven, CT; 3) Dept Psychiatry, Indiana Univ, Indianapolis, IN.

The proteolipid protein gene, PLP, encodes the major protein of myelin of the central nervous system. Duplications of the PLP gene have been observed in patients with Pelizaeus-Merzbacher disease (PMD), an X-linked neurodegenerative disease. Duplication analysis of five affected males and their carrier mothers has been reported using dual-color probes of both the PLP gene and alpha satellite region of the X chromosome[1]. In contrast to this published report, we have established two-color interphase FISH analysis for the PLP gene duplication in our cytogenetic laboratory with a cosmid for Xq rather than an X alpha satellite probe. The PLP probe, cosmid 350.1 was labeled with digoxigenin and a 40 kb cosmid, cos 9, was labeled with biotin. Both probes were hybridized to slides with fixed cells of established lymphoblastoid cell lines from 32 PMD family members (7 females and 25 males). One hundred nuclei for each sample were scored on a grid comparing the number of green (1-2) with the number of red (1-4) signals. Duplication mutations were detected in 44% of these individuals. Four females were duplication carriers and 10 affected males were carriers. It is now clear that PLP gene duplication is a significant mutation in the etiology of Pelizaeus-Merzbacher disease.

[1] Woodward K, Kendall E, Vetrie D, Malcolm S. Pelizaeus-Merzbacher Disease: Identification of Xq22 Proteolipid-Protein Duplications and Characterization of Breakpoints by Interphase FISH. Am J Hum Genet 63:207-217, l998.

Program Nr: 2044 from the 1999 ASHG Annual Meeting

A rare dicentric chromosome 22 variant detected during prenatal diagnosis in three unrelated pregnancies. *V. Velissariou[1], A. Hatzipouliou[1], S. Christopoulou[1], J. Donohue[1], K. Bakou[1], M.B. Petersen[1], P. Grigori[2], E. Lyberatou[2].* 1) Dept of Genetics and Molecular Biology, "Mitera" Maternity Hospital, Athens, Greece; 2) Dept of Genetics, "Alexandra" Hospital, Athens, Greece.

A chromosome 22 with extra chromosomal material in region 22q11 was detected in three different, unrelated pregnancies during prenatal diagnosis. The extra genetic material was shown to be a tandem duplication of the centromeric region and the satellites, using conventional cytogenetic techniques and FISH analysis. The variant chromosome 22 was inherited in all three cases from one of the parents and was not associated with any clinical abnormality.

Program Nr: 2045 from the 1999 ASHG Annual Meeting

Screening for chromosome abnormalities in spermatozoa from infertile men in a clinical setting. *F. Vidal[1], J. Blanco[1], M.C. Rubio[2], B. Aran[3], S. Egozcue[1], R. Alcolea[4], C. Simn[2], A. Veiga[3], Y. Mnguez[2], J. Remoh[2], A. Pellicer[2], P.N. Barri[3], J.M. Vendrell[3], S. Marina[4], J. Egozcue[1]*. 1) Cell Biology, Univ Autonoma de Barcelona, Barcelona, Spain; 2) IVI, Valencia,Spain; 3) Institut Dexeus, Barcelona, Spain; 4) CEFER, Barcelona, Spain.

Since the use of fluorescent in situ hybridization (FISH) techniques in decondensed sperm nuclei, several groups have undertaken the study of aneuploidy and diploidy levels in spermatozoa from infertile men. From june 1996, spermatozoa FISH analysis in infertile men showing moderate to extremely impaired semen parameters, most of them referred as ICSI candidates, have been incorporated as an adjunct to the standard genetic screening suggested in these patients. In this paper we report our results in a series of 57 patients studied during this period. Sperm samples from 5 normal fertile donors served as a control group. In all cases, semen parameters were evaluated and ejaculated spermatozoa were collected and fixed for FISH analysis. Disomy and diploidy frequencies, using triple color FISH with centromeric DNA probes for chromosomes X, Y and 18 and dual color FISH with locus specific probes for chromosomes 13 and 21, have been evaluated. Our results are in concordance with previous reports from series including lower number of patients and significantly higher incidence of sex chromosome disomy and of diploid spermatozoa were observed in the infertile patients. Furthermore, infertile patients have been delineated into specific subgroups according to their semen parameters. Patients with severe oligoasthenoteratozoospermia reflected higher disomy and diploidy frequencies. Detailed results obtained in each subgroup will be presented and discussed. Our results confirm that FISH studies to estimate aneuploidy an diploidy levels in human sperm seem to be indicated in ICSI candidates and that this approach is feasible in a clinical setting.

Program Nr: 2046 from the 1999 ASHG Annual Meeting

Familial case of inversion in chromosome 7 with break-point within alphoid DNA in child with mesodermal displasia, ectrodactyly and growth deficiency: Is it an example of position effect variegation in man? *S.G. Vorsanova[1], Y.B. Yurov[1,2], A.D. Kolotii[1], E.S. Useinova[1], V.V. Monachov[2], M.S. Strachova[1], A.A. Tcheburkin[1]*. 1) Molecular Cytogenetics, Institute of Pediatrics, Moscow, Russia; 2) National Centre of Mental Health,RAMS, Moscow, Russia.

Familial case of chromosomal inversion (inv(7)(p11q21.1) was analyzed by conventional cytogenetic and molecular-cytogenetic methods. Inversion segregated from phenotypically normal father to his daughter and son. Boy has severe phenotypic abnormalities, including mesodermal dysplasia, ectrodactyly, growth and sexual development retardation, chicken breast, osteoporosis, gothic oral ark, short neck, deformations of ears, whereas his sister was healthy. Clinical picture in affected boy is similar to atypical form of EEC syndrome (MIM 129810). The breakpoints was detected by GTG and FISH using centromeric alphoid DNA probe and site-specific PAC probes from the short and long arm of the chromosome 7. One break-point was situated in alphoid DNA from the long arm distal of active centromere, and second at 7q21.1 in region of the locus of EEC syndrome. Therefore, alpha-satellite DNA was presented in two separate blocks-one is situated around the active centromere at the region of primary construction and second - in the short arm. New disposition of heterochromatic DNA leads to unusual junctions of euchromatic DNA from both short and long arm with alphoid satellite DNA. Variable phenotypic appearance in three individuals with inv7 could be, probably, related with variable expression of the genes, which are located near heterochromatic DNA. This familial case of inversion with break-points within heterochromatin and variable phenotypic appearance could be explained by phenomenon of position effect variegation, which is not yet discovered in man. Supported in parts by Russian Human Genome Program.

Program Nr: 2047 from the 1999 ASHG Annual Meeting

Clinical applications of PRINS: evaluation of X-Y interchange and AZF deletion in an azoospermic male. *S.S. Wachtel[1], J.S. Kadandale[2], R.S. Wilroy[3], Y. Tunca[2], P.R. Martens[3], A.T. Tharapel[2]*. 1) Reproductive Genetics Laboratory, Dept Obstetrics & Gynecology, Univ Tennessee, Memphis, TN; 2) Clinical and Molecular Cytogenetics Laboratory, Depts Obstetrics & Gynecology and Pediatrics, Univ Tennessee, Memphis, TN; 3) Dept Pediatrics, Univ Tennessee, Memphis, TN.

Nearly 15% of azoospermic men have microdeletions in Yq. Atypical cases involve 45,X/46,XY mosaics with a nonfluorescent Y chromosome. We used primed in situ labeling (PRINS) to delineate a chromosomal rearrangement in an azoospermic male with a mosaic karyotype and an Xp;Yp translocation. The patient was a 27-year-old man referred for infertility. Examination revealed standard height (172.2 cm) and unremarkable external genitalia; the testes were normal-appearing and soft. The karyotype in blood lymphocytes was 45,X/46,der(X)t(Xp;Yp),+mar (<10% of cells were 45,X). Translocation was ascertained by FISH with dual color X and Y paint probes, and confirmed by PRINS with locus-specific primers. By use of FISH, several individual loci were identified on the X chromosome, including STS, DMD, DXZ1, AR, XIST and subtelomeric Xp and Xq. On the Y chromosome, AMELY, DYZ3, DYZ1 and subtelomeric Yp were identified, but subtelomeric Yq was not. Yq contains AZF (azoospermia factor), a region of 4-5 Mb subsuming the RRM (RNA recognition motif) gene family, implicated in normal spermatogenesis. Using PRINS, we identified SRY on Xp and noted absence of two genes in the AZF region: RRM1 and RRM2. Our results confirm (i) X-Y interchange and (ii) deletion within the Yq-situated AZF region. The karyotype was accordingly redesignated: 45,X/46,t(X;Y)(p22.3;p11.2),del(Y)(q11.2). We infer that PRINS, combining features of FISH and PCR, is a valuable diagnostic tool with potential applications in the study of unusual chromosome rearrangements such as the one described here.

Program Nr: 2048 from the 1999 ASHG Annual Meeting

A familial interstitial deletion of 21q. It is possible for a recessive gene responsible for mental retardation to exist within 21q11-21. *K. Wakui[1], T. Kubota[1], E. Hidaka[2], M. Ishikawa[2], T. Katsuyama[2], Y. Fukushima[1]*. 1) Dept Hygiene & Med Genetics, Shinshu Univ Sch Medicine, Matsumoto, Japan; 2) Dept Lab Med, Shinshu Univ Sch Medicine, Matsumoto, Japan.

We report a family including a mother of normal intelligence and two children with mild mental retardation. All these family members had the same interstitial deletion of 21q11.2-q21.3. A four-year-old first daughter of the couple experienced deafness, strabismus and mild mental retardation. As the mother had suffered from rubella during pregnancy, the daughter had been thought to have congenital rubella syndrome. However, chromosome analysis revealed that she had an interstitial deletion of 21q. Parental karyotyping showed that the mother also had the same deletion of 21q. The mother was phenotypically and mentally normal. Any extra signals were not observed on her other chromosomes by FISH analysis using whole chromosome painting 21 probe. A one-year-old second daughter was healthy, mentally normal and had normal karyotype. The first son had been diagnosed as having 21q monosomy by prenatal diagnosis. But the parents wanted to continue the pregnancy, and the mother was delivered of him at 39 weeks gestation. His birth weight was 2900 g. He had no deafness or malformations, but developmental delay was noticed at 4 months. High resolution chromosome analysis revealed that these three members had the same deletion of 21q, and the breakpoints were 21q11.2 and 21q21.3. The extension of the cytogenetically visible deletion of these three members was defined by analyzing those genomic DNA with highly polymorphic DNA markers located on the proximal region of 21q (cen-D21S215-D21S1433-CHLC.GATA22H11-D21S1435-D21S1442-D21S261-D21S1909-D21S1898-D21S1920-D21S1921-CHLC.GATA45C03-D21S1444-tel). Molecular analysis with these markers demonstrated that these three members had the same deletion of 21q. The proximal breakpoint of the deletion was located between D21S215 and D21S1433 and the distal breakpoint was located between D21S1442 and D21S261. These clinical and molecular findings suggest that it is possible for a recessive gene responsible for mental retardation to exist within 21q11-21.

Program Nr: 2049 from the 1999 ASHG Annual Meeting

Chromosomal abnormalities in autism. *T.H. Wassink[1], J. Piven[1], V.C. Sheffield[2], S.E. Folstein[3], J.L. Haines[4], S.R. Patil[2]*. 1) Department of Psychiatry, University of Iowa Hospitals and Clinics, Iowa City, IA; 2) Department of Pediatrics, University of Iowa Hospitals and Clinics, Iowa; 3) Department of Psychiatry, New England Medical Center, Boston, MA; 4) Program in Human Genetics, Vanderbilt University Medical Center, Nashville, TN.

A wide range of chromosomal abnormalities have been reported in subjects with autism. The estimated frequency of such abnormalities varies from 1 to 15%. We report here our series of cytogenetic examinations of subjects with autism, and a related examination of micro-deletions and duplications on the 15q11.2-q13 region. From 1989 to 1999, there were 898 subjects diagnosed with autism through the University of Iowa Child and Adolescent Psychiatry Clinic. Of these, 264 (29%) were referred for cytogenetic analyses. Referrals were not controlled, but rather were based on clinical judgement. Of subjects who were referred for cytogenetics, 25 (9.6%) had an identifiable cytogenetic abnormality. The most common abnormality was fragile X, found in 6 subjects (2.3%). Five subjects (1.9%) had some other form of sex chromosome abnormality, and the remaining 14 (5.3%) had an autosomal abnormality. Of these, 2 had Trisomy 21 (0.8%), and 5 (1.9%) had an abnormality involving the 15q11.2-q13 region. Because of the prevalence of the chromosome 15 abnormalities, and the broader interest in 15q11.2-q13 in autism, we also examined 12 polymorphic markers spanning this region in 49 autism trios, looking for deletions and duplications of parental alleles in the offspring. Approximately 8% of the trios were found to have such abnormalities, the most common being duplication of maternal alleles. Taken together, these data may help to focus the search for autism disease genes, and provide further support for 15q11.2-q13 as a region of interest in autism.

Program Nr: 2050 from the 1999 ASHG Annual Meeting

Interstitial 1p deletion in an infant arising from a unique paternal karyotype. *L.M. Welter-Dahl, J. Ahmad, S. Herath, J.L. Andeweg, T. Barnhart, M. Engel, C.A. Curtis, R.R. Higgins.* Allina Cytogenetics Laboratory, Abbott Northwestern Hospital, Minneapolis, MN.

Cytogenetic studies were requested on a one day-old infant to rule out Down syndrome. Phenotypic findings of the newborn included: unusual eyes, prominent palmar creases, prominent nasal bridge, long creased ears, and hypospadias. Chromosome analysis revealed a 46,XY karyotype with an interstitial deletion in one chromosome 1 involving G-band region 1p11-1p13.3 [46,XY,del(1)(p11p13.3)]. Both parents are phenotypically normal. Parental cytogenetic studies were subsequently done. The mother's karyotype is 46,XX. The father has a mosaic karyotype with two cell-lines: one male cell-line [4 cells] with the deleted chromosome 1 previously identified in the child and a second male cell-line [16 cells] with the deleted chromosome 1 as well as a small ring-shaped chromosome. FISH analysis using a chromosome 1-specific painting probe established that the ring chromosome originates from chromosome 1. The ring chromosome also stains weakly positive following CBL-staining. We speculate that the r(1) chromosome contains the DNA sequences 1p11-1p13.3 that are absent in the del(1), resulting in a paternal karyotype of mos 47,XY,del(1)(p11p13.3), +r(1)(p11p13.3)[16]/46,XY,del(1)(p11p13.3)[4]. Such chromosome rearrangements are very rare and this appears to be the first such case involving the 1p arm.

Program Nr: 2051 from the 1999 ASHG Annual Meeting

Molecular cytogenetic identification and characterization of marker and derivative chromosomes. *G.D. Wenger, A. Thomas, J. Labanowska.* Laboratory Medicine, Children's Hosp, Columbus, OH.

Identification of the origin of additional genetic material in marker and derivative chromosomes is of importance for clinical management and establishment of karyotype-phenotype correlations. We have used a variety of molecular cytogenetic techniques and reagents to identify and characterize marker and derivative chromosomes identified by high resolution cytogenetic analysis, including a satellite probes, unique sequence probes including subtelomeric probes, whole chromosome paints, Cytocell Multiprobe (Rainbow Scientific) devices, and comparative genomic hybridization (CGH). In each case, the additional material was not readily identified by GTG-banding analysis, but was fully identified by molecular cytogenetic techniques. We have examined 10 cases with derivative chromosomes, including 3 *de novo* cases and one in which the supernumerary derivative chromosome is apparently the result of 3:1 segregation. Three cases were found to have duplication of sequences within the same chromosome; CGH was successful in evaluating the duplicated material in 2 of these. Supernumerary marker chromosomes were identified in 6 cases, each with 1-3 markers and all apparently *de novo*. These include one case with a small marker of cs 15 origin and uniparental disomy for cs 15 resulting in diagnosis of Prader-Willi syndrome, and one case with an asymmetrical bisatellited cs 22 marker for which investigation of the marker was conducted in buccal smears and hair follicles as well as peripheral blood. The ease of identification of the additional material varied with the size and nature of the material; CGH was limited in sensitivity and resolution. These strategies are particularly important in characterization of *de novo* rearrangements, or in cases in which study of both parental karyotypes is not possible. These studies indicate that careful choice of molecular cytogenetic reagents and techniques, applied in a systematic fashion, provides efficient characterization of subtle chromosome aberrations, the results of which can aid in clinical management and contribute to the cytogenetic/clinical genetics literature in terms of karyotype-phenotype correlations.

Program Nr: 2052 from the 1999 ASHG Annual Meeting

Partial trisomy 16q in a full term infant, mosaic for an unbalanced translocation, der(19)t(16;19)(q13;q13). *H.E. Wyandt, X.L. Huang, J.M. Milunsky.* Ctr Human Genetics, Boston Univ Sch Medicine, Boston, MA.

A 2.935 kg infant with ambiguous genitalia and multiple congenital abnormalities was delivered, by cesarean section, to a 36-y-o Hispanic female. Family history was non-contributory. The pregnancy was complicated by oligohydramnios and a small-for-gestational-age fetus. Apgars were 3, 7 and 8 at 1, 5 and 10 min, respectively. An echocardiogram showed PDA and mild isthmic hypoplasia. Weight was in the 40th, length in the 10th and head circumference in the 75th percentiles. Anomalies included low-set, posteriorly rotated ears with over-folded helices, broad nasal bridge, epicanthal folds, high-arched palate, short, webbed neck, barrel chest with wide-spaced nipples, bifid scrotum, small phallus and 4th-degree hypospadius. Extremities, especially the feet, showed lymphedema. Fingers were tapered with hyperconvex nails and contractures of PIP joints. Fifth finger clinodactyly was present. The feet were small with increased distance between the first and second toes. The infant had hypotonia and probelms sucking and feeding. BAERS revealed moderate hearing loss. Study of peripheral blood revealed 70% of cells with a translocation between chromosomes 16 and 19, with telomeric loss only from the terminal band of 19q and parital trisomy for most of 16q(q13-qter), confirmed by chromosome painting. Thirty percent of of cells had a normal male karyotype. Maternal chromosomes were normal; father was not available. A comparison is made of the findings in our patient with those reported.

Program Nr: 2053 from the 1999 ASHG Annual Meeting

Evidence of a locus for cardiac development on mouse chromosome 13C3.4-D1.5. *K.L. Wydner[1], J. Zhang[2], T. Williams[2], D. Sciorra[1], L.J. Sciorra[1]*. 1) Dept OB/GYN, Diagnostic Genetics, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ; 2) Dept. Molecular, Cellular, & Developmental Biology, Yale University, New Haven, CT.

Normal development requires a series of finely orchestrated processes in which intact genetic information plays a critical role in determining cell viability, function, and fate. Transcription factors are important in the regulation of cellular gene expression, operating at specific target sequences or in regions of promotors and enhancers. Interruption of normal transcription factor activity can result in aberrant development or lead to disease. Using a 40 kb portion of the human transcription factor AP-2a gene fused to a 3.8kb lac Z reporter, a transgenic mouse strain was generated by pronucleus injection of fertilized mouse eggs. Random integration of the transgene resulted in an altered cardiac phenotype in the transgenic mice. Expression of the lac Z was observed in early developing heart tissue (E8.0 - E11.0 mouse embryos) in addition to where the endogenous AP-2a is expressed (trigeminal ganglia, limb buds, and facial mesenchyme). To aid in the discovery of gene(s) involved in the generation of this phenotype, we performed FISH on high resolution metaphase chromosomes prepared from transgenic mouse embryo fibroblasts. Utilizing a digoxigenin-labelled 13 kb genomic probe and conventional FISH methods, the site of the AP-2a transgene was localized to the 13C3.4-D1.5 region of DAPI banded mouse chromosomes. Interestingly, dual color FISH experiments with a biotinylated mouse cosmid probe Tcfap2 (Warren et al., Genomics, 1996) confirmed the assignment of the transgene to mouse chromosome 13 clearly below the 13A5-B1 location of the endogenous sequence. The non-endogenous localization of the transgene suggests 13C3.4-D1.5 is a cytogenetic location of mouse sequences which somehow influence cardiac development. Physical mapping of the transgene sequence to this region of mouse chromosome 13 in these mutant animals provides the groundwork for future studies to clone and ultimatley identify the sequence(s) of gene(s) involved in the creation of the aberrant phenotype.

Program Nr: 2054 from the 1999 ASHG Annual Meeting

Direct duplication of 4p14p15.31. *J. Xu[1,2], V. Freeman[1,2], M.J.M. Nowaczyk[1,2,3].* 1) Department of Pathology & Molecular Medicine, McMaster University, Hamilton; 2) Hamilton Regional laboratory Medicine Program, Hamilton; 3) Department of Pediatrics, McMaster University, Hamilton.

Delineation of a cytogenetic duplication and its associated clinical features has potential in physical mapping of phenotype, understanding gene dosage effect, or identifying genes interrupted by the breakpoints. We report a de novo direct tandem duplication of 4p14->p15.31 in a boy with congenital anomalies. The boy was born at full term following an uncomplicated pregnancy to a 34-year old G7P5SA1. At six months of age he was referred for genetic investigation because of poor growth, hypotonia, and developmental delay. On physical examination at 10 months, his weight was at the 3rd centile. He had mild dysmorphic facial features, including deep set eyes, bitemporal narrowing, small, beak-like nose, and thin and sparse scalp hair. There was hypoplasia of terminal phalanges of fingers, fifth finger clinodactyly, ulnar deviation of hands.

Chromosome analysis showed the presence of a duplication in the proximal short arm of chromosome 4:46,XY,dup(4)(p14p15.31). This duplication is confirmed to consist of only chromosome 4 material using whole chromosome painting. Parental chromosomes were apparently normal.

Approximately 40 cases of duplication 4p have been reported. Most of these cases are mixed duplication/deletion derived from translocations and involve the distal half of 4p. Main features of 4p duplication patients include large and low-set ears, microcephaly, a prominent glabella, a bulbous nose, growth deficiency, severe mental retardation, seizures, scoliosis and 5th finger clinodactyly.

To our knowledge, this is the first case of "pure" trisomy of 4p14->p15.31. Clinical follow-up of our patient and a further molecular characterization of the breakpoints involved should shed light on the developmental effect of the duplicated genes in this region.

Program Nr: 2055 from the 1999 ASHG Annual Meeting

A novel 21p+ chromosome with duplication of Down syndrome critical region in the amniotic fluid cells of a second trimester pregnancy. *A.K. Yenamandra[1], L. Trinchitella[1], G. Haberman[1], R. Perrone[1], C. McKenna[2], M. Bialer[2], P. Koduru[1]*. 1) Dept. Path/Lab Cell Genetics; 2) Dept. Pediatrics/ Div. Human Genetics, North Shore Univ. Hospital, 300 Community Drive, Manhasset, NY 11030.

The majority of Down syndrome cases are due to complete trisomy of chromosome 21 or the long arm (q) of 21. Much less frequently it is caused by partial trisomy 21 resulting from a duplication which is associated with certain features of Down syndrome phenotypes, with the region 21q22.13-22.2 having prominent influence. These infants will have the typical manifestations like hypotonia, brachycephaly, flat facial profile, upslanting palpebral fissures, epicanthal folds, protruding tongue, redundant nuchal skin, fifth finger clinodactyly, and abnormal palmar creases. We report on a second trimester female fetus detected at 21.5 weeks gestation with bilateral ventriculomegaly. Cytogenetic analysis of amniotic fluid cells revealed an abnormal karyotype of 46,XX, 21p+. The 21p+ chromosome had a very large p arm and had a different banding pattern than the q arm. This region was negative with C-banding and silver stain. Chromosome analysis of PHA stimulated blood from both parents revealed normal karyotypes. Therefore, the 21p+ chromosome was considered as a de novo event. Chromosome 21 specific painting fluorescence probe WCP 21 (Vysis Inc.) completely painted this chromosome indicating that the additional material on the 'p' arm was derived from a partial duplication of the long arm. This was confirmed by hybridization with a Down syndrome critical region FISH probe LSI 21 (Vysis Inc.) which showed two pairs of distinct signals one on either side of the centromere in the 21p+ chromosome and only one pair of signals in the normal 21 homologue. These results suggest that the fetus is trisomic for Down syndrome region. A fetal echocardiogram revealed two small VSD's. A comprehensive fetal ultrasonographic study was recommended to rule out additional clinical features. Parents were counseled about Down syndrome and were informed that hydrocephalus is not seen at increased incidence in Down syndrome. The couple decided to continue the pregnancy which is currently in progress.

Program Nr: 2056 from the 1999 ASHG Annual Meeting

Molecular and FISH analysis of a chromosome 8p duplication syndrome case study. *J.H. Youngblom, G. Helbig, J.J. Youngblom.* Biology Dept., California State University-Stanislaus, Turlock, CA.

We present a new case involving a 4-year-old girl with an inverted duplication of chromosome 8p. The clinical presentation of this syndrome is variable in patients due to differences in the duplicated and deleted regions. However, consistent features in all reported cases include severe mental retardation and speech disorder. Some patients, as in this case, also have autism. Fluorescence in situ hybridization (FISH) analysis was conducted on this patient to ascertain the specific regions involved in the duplication and deletion. This process is essential for all individuals with the syndrome in order to identify the common critical region associated with the classical symptoms of the disorder. PCR was used to generate a biotin labeled DNA probe representing the tip of chromosome 8p23.1-.3. Results of the FISH analysis using this probe showed that the patient has a partial duplication of this region. The duplicated segments are not situated tandemly, but are separated by a small non-labeled region. The proximal segment is smaller than the distal segment. The distal segment appears to be of normal size, indicating that any deletion at the terminal end must be quite small. Additionally, the probe had a high homology to the centromeric regions on chromosome 1, and to a lesser extent chromosome 7. This observation was also noted in the control sample. To delineate the region of the terminal deletion on the chromosome, we used a polymorphic dinucleotide repeat marker, designated D8S201. This marker is one of the most terminal markers on the 8p chromosome available to date and has been mapped to 8p23.2. DNA isolated from cheek samples donated by the mother, father, and the patient were used to amplify this region by PCR. The PCR products were run on a 6% denaturing polyacrylamide gel. Each of the parents was heterozygous for different alleles. The child unequivocally showed the presence of both alleles, one from each parent, thereby indicating that the deletion is distal to the 8p23.2 region. This finding is in contrast to virtually all other reported cases of this condition, which have shown the deleted segment includes the 8p23.2 region.

Program Nr: 2057 from the 1999 ASHG Annual Meeting

Double chromosome abnormalities-- mosaic monosomy 13 and extra marker iso(22q) in a new born. *C.W. Yu[1], H-G. Bock[1], M. LeBlanc[2], R.E. Shenefelt[3]*. 1) Dept. of Preventive Medicine; 2) Dept. of Pediatrics; 3) Dept. of Pathology, Univ. of Mississippi Medical Ctr., Jackson.

Patient BGM was a 37 week new born to a 40 year old G6P5 black female. The birth weight was 2,325g, birth length 49cm, and head circumference 34.5cm. Mother had PIH, polyhydramnios, and non-reactive NST during pregnancy. Patient was admitted to hospital due to congenital anomalies and failure to thrive. Physical examination showed a soft and flat fontanelle, opacity of the right eye, epicanthal fold, small nose, smooth and flat philtrum, micrognathia, bilateral ear tag, preauricular pits, and widely spaced nipples.

Cytogenetic analysis was performed from 72 hour PHA stimulated peripheral blood cultures. GTG banding demonstrated that all cells had a small, extra bisatellited marker chromosome. Four of the fifty metaphases examined also had monosomy 13. FISH studies using alpha-satellite DNA probes and unique sequence DNA probes confirmed the marker chromosome was a bisatellited, dicentric isochromosome 22 with breakpoint at 22q11.2, containing the DiGeorge syndrome/ velocardiofacial syndrome (DGS/VCFS) region. Patient expired at the age of 22 days. Postmortem tissue from heart, lungs, kidneys, and skin all had the marker chromosome. Parental blood chromosome studies were normal.

The presence of three or four copies of proximal 22q11.2, including at least the locus D22S57, is the most common molecular finding of cat-eye syndrome (CES). Our patient had mild features of CES which may be attributed to the presence of a monosomy 13 cell line. FISH studies indicate this marker has a breakpoint distal to locus TUPLE1, possibly including locus D22S941. A region of overlap on 22q11.2 for genes responsible for CES and DGS/VCFS suggests the presence of dosage sensitive genes in this interval.

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Program Nr: 2058 from the 1999 ASHG Annual Meeting

Tourette syndrome in two brothers with balanced and unbalanced chromosome 12 rearrangements. *J. Yu[1], X. Guan[2], B. Pober[1], T. Yang-Feng[1]*. 1) Genetics Dept, Yale Univ Sch Medicine, New Haven, CT; 2) Hong Kong University, Hong Kong.

A 9 year old boy was referred for genetics evaluation because of minor dysmorphic features and developmental delay. Medical problems included: recurrent otitis media, strabismus, mild motor and speech delays and Tourette syndrome (TS) diagnosed at age 5 years requiring treatment with Tenex. Pertinent physical findings were: low set ears, narrow palate and mild joint hyperextensibility. Family history was remarkable for a 4 year old brother with tics considered probable TS. Parents, brother and a sister are all developmentally normal; there is no family history of TS. Cytogenetic study on the proband revealed an increased size of band 12q15. Paternal karyotype was normal while maternal karyotype demonstrated a balanced intrachromosomal insertion [ins(12)(q15p12p11.2)] which was also present in the brother and sister. The proband thus appeared to have a duplication of 12p11.2-p12 inserted into band 12q15, possibly resulting from crossing over between the maternal normal and rearranged chromosomes 12. The proband's karyotype is designated as: 46,XX,rec(12)dup(12)(p11.2p12)ins(12)(q15p12 p11.2)mat. These findings were further confirmed by FISH using a chromosome 12 painting probe and the microdissected probe spanning breakage/reunion breakpoints in 12q15 of maternal ins(12). Both the proband and his brother have TS, however, the rearranged 12 is unbalanced in the proband but apparently balanced in his brother. Of further interest is that 2 female family members carry the presumably same balanced ins(12) but have no evidence of TS. TS has been considered a sex-influenced autosomal dominant disorder as 3/4 patients are male; multiple disease loci appear possible. This family suggests a link between TS and chromosome 12 particularly in the regions of insertion breakpoints, although coincidental occurrence of these two findings cannot be excluded. Additional phenotypic abnormalities in the proband are likely associated with the duplication of the 12p11.2-p12 region.

Program Nr: 2059 from the 1999 ASHG Annual Meeting

Direct evidences for skewed X-chromosome inactivation at Rett syndrome by combination of FISH and BrdU+Hoechst staining techniques. *Y.B Yurov[1,2], S.G. Vorsanova[1,2], A.D. Kolotii[2], V.O. Sharonin[1,2], I.V. Soloviev[1].* 1) National Centre of Mental Health, RAMS, Moscow, Russia; 2) Institute of Pediatrics and Children Surgery MH, Moscow, Russia.

Rett syndrome (RS) is a developmental disorder, affecting girls. The aetiology of RS is unknown, but genetic factors are important. RS is commonly thought of as an X linked dominant disorder lethal to hemizygous males. However, at present time its biological and genetic basis remains obscure. There is one remarkable finding in cytogenetic studies of RS, indicating on specific alterations in replication pattern of inactive X chromosome in girls with RS. X inactivation is thought to be a random process with 50%:50% ratio for maternal and paternal chromosomes X. However, for some X-linked disorders, non-random X-inactivation could take place leading to completely skewing of X-inactivation and selection against cells in which the mutant gene is on the active X chromosome. We have performed cytogenetic studies to establish that skewed X inactivation could take place at RS. In preliminary FISH studies using centromeric alphoid DNA probe we have detected three RS girls from 20 studied with heteromorphism of homologous chromosomes X on amount of alphoid DNA. This phenomenon allows us to clearly differentiate maternal and paternal X-chromosomes by FISH in metaphase cells of these girls. Step-wise application of BrdU + Hoechst staining to identify early (active) and late (inactive) replicating chromosomes X and FISH technique to clearly differentiate two homologous chromosomes X allows directly determine the origin of inactive chromosome X. We have detected non-random X-inactivation in RS patients with preferential inactivation of one X chromosome over the other X chromosome in 100% of cells of two RS girls and 70% cells of the third girl. Therefore, RS similarly to X-linked diseases, could be characterized by skewed X inactivation with variable penetrance in peripheral blood cells. Supported in parts by INTAS and IRSA grants.

Program Nr: 2060 from the 1999 ASHG Annual Meeting

Prenatal Diagnosis of Trisomy 4 Mosaicism. *A. Zaslav[1], D. Blumenthal[2], J. Willner[3], G. Pierno[4], J. Fox[2].* 1) Dept. of Pathology, Long Island Jewish Medical Center, The Long Island Campus of The Albert Einstein College of Medicine, New Hyde Park, NY; 2) Dept. of Pediatrics Schneiders Childrens Hospital, The Long Island Campus of The Albert Einstein College of Medicine, New Hyde Park, NY; 3) Dept. of Human Genetics, Mount Sinai School of Medicine, New York, NY; 4) Sonoscan Genetic Sciences, Forest Hills, NY.

Trisomy 4 mosaicism is rare. We present a prenatally diagnosed case of trisomy 4 mosaicism. Amniocentesis was performed for AMA. The karyotype was 47,XY,+4[3]/46,XY[33]. Trisomy 4 was found in three cells from two different vessels, indicating true mosaicism. A high-resolution US and fetal echo were performed at 23 wk and neither revealed abnormalities. The infant was delivered at 37 wk. Apgar scores were 9 and 9 at 1 and 5 min. The infant had a normal examination. Chromosome analysis of 150 cells from cord and peripheral blood, foreskin and umbilical cord revealed 46,XY in all cells. There was no evidence of trisomy 4. Only 2 prenatally diagnosed cases of mosaic trisomy 4 have been reported. One resulted in a live born male who was developmentally and physically normal at one year. Normal cells were found in PUBS and foreskin (Hsu, et al., 1997, Prenat Diag 17:201). The other resulted in an abnormal live born female with multiple congenital anomalies, but no significant developmental delay at one year (Marion, et al., 1990, Am J Med Genet 37:362). PUBS and peripheral blood were normal. Trisomy 4 was found in placental and bilateral forearm biopsies. The absence of trisomy 4 cells postnatally in our patient indicates that the trisomy 4 mosaicism was most likely placental. Prenatally diagnosed chromosome mosaicism presents a diagnostic dilemma for both patient and clinician. This is only the third report of trisomy 4 mosaicism. Additional cases of prenatally diagnosed mosaicism for rare trisomies are necessary to accurately access the significance of these findings.

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Program Nr: 2061 from the 1999 ASHG Annual Meeting

High throughput mapping of Cri du Chat deletions on 5p using comparative genomic hybridization to DNA microarrays. *X. Zhang[1], R. Segraves[1], L. Bolund[2], H.M. Yang[2], E. Niebuhr[3], J. Gray[1], D. Albertson[1], D. Pinkel[1]*. 1) Cancer Center, UCSF, San Francisco, CA; 2) Inst. of Human Genetics,Aauhus University, Danmark; 3) Medical Genetics, University of Copenhagen, Copenhagen, Denark.

Cri du Chat is caused by 5p deletions, with the detailed phenotype dependent on the deleted region. High resolution mapping of the deletions will permit localization of genes that may be associated with the phenotype. We have developed a microarray form of comparative genomic hybridization, array CGH, that permits reliable detection of these deletions. The array elements consist of genomic BAC and P1 clones, each containing a mapped STS or EST, spaced at approximately 1 Mb intervals along 5p. A higher density of elements was used for the established critical area at 5p15.2, as well as at 5p13. Hybridization employed 400 ng each of specimen and normal reference genomic DNA labeled with fluorescein and Alexa 568 respectively. Fluorescence data for each fluorochrome was obtained from the entire array in a few seconds using a custom designed CCD imaging system. Illumination was supplied by a mercury arc lamp. Fluorescence ratios for diploid targets were 1.0 +/- 0.07 while ratios on deleted clones were 0.55 +/- 0.05 (mean +/- s.d.). This measurement precision is expected to permit recognition of deletions with false positive and negative rates below one in several thousand. One clone was found to have a ratio of 0.75 when included in some deletions. FISH mapping showed that this clone hybridized to two locations on chromosome 5p, indicating its probable duplication in the normal genome. Thus copy number of this clone decreased from 4 to 3 copies in the deletions. Array CGH analysis of 91 Cri du Chat cases will be presented. The measurement precision obtained in this work indicates that arrays containing clones that map to established loci involved in deletion/duplication syndromes plus clones at regular intervals throughout the genome could be used to provide a rapid high resolutions diagnostic capability for constitutional aberrations. Work supported by NICHD RO1 HD 17665 and Vysis Inc. ±±±±±±±.

Program Nr: 2062 from the 1999 ASHG Annual Meeting

Telomere-telomere fusion of chromosomes 7 and 22 with an interstitial deletion of chromosome 7p11.2-p15.1: phenotypic consequences and possible mechanisms. *S.M. Zneimer, S.D. Stewart.* Cytogenetics Laboratory, SmithKline Beecham Clinical Laboratories, Van Nuys, CA.

We report a rare, if not unique, case of a telomere-telomere fusion of chromosomes 7 and 22 as well as an interstitial deletion of chromosome 7p11.2p15.1 in a newborn with congenital anomalies. This newborn presented for chromosome analysis with bilateral cataracts, distal limb abnormalities and renal dysmorphogenesis. Chromosome analysis showed 45,XY,-7,der(22)psudic(7;22)(p22;p13)del(7)(p11.2p15.1)[20]. This short arm to short arm fusion of chromosomes 7 and 22 results in a psuedodicentric chromosome. C-banding confirms the presence of 2 centromeres with the active centromere derived from chromosome 22. In addition, the chromosome also appears to have a deletion of chromatin from bands p11.2 to p15.1. Parental blood chromosome analysis revealed normal karyotypes. Fluorescence in situ hybridization studies are underway to better characterize this complex rearrangement. Loss of genetic material in this region of chromosome 7p has been implicated in the pathophysiology of craniosynostosis syndromes, cephalosyndactyly syndrome and ocular manifestation syndrome. Given the number of reports in the literature describing syndromes associated with deletions in chromosome 7p, this region of the genome may be predisposed to deletions/duplications due to misalignment of conserved homologous sequences. It is also a region involving at least 3 different syndromes described, suggesting contiguous genes with microdeletion syndromes. The few reports in the literature describing telomere fusions have been associated with a neoplastic process. There is a paucity of information describing telomere fusions which are constitutional in origin, let alone in combination with an interstitial deletion causing clinical consequences. Telomere fusions have been shown to be a part of chromosome replication by the formation of "hairpin" 5' to 3' covalent binding, and since telomeres contain repeated short base pair sequences, it is possible to fuse two telomeres with a lack of fidelity and loose DNA sequences in the process causing deletions near the telomeres.

Program Nr: 2063 from the 1999 ASHG Annual Meeting

Clinical diagnostic criteria for Wolf-Hirschhorn syndrome. *M. Zollino[1], C. Di Stefano[1], G. Zampino[2], L. Bortotto[3], T.J. Wright[4], M.R. Altherr[5], G. Neri[1].* 1) Genetica Medica, Univ Cattolica, Rome, Italy; 2) Istituto di Pediatria, Univ Cattolica, Rome Italy; 3) Ospedale S.M.M., Udine, Italy; 4) Dept.of Neurobiology and Anatomy, Univ of Utah, Salt Lake City, UT; 5) Genomics group,Life Sciences Division, Los Alamos Natl Lab, Los Alamos.

Eighteen Wolf-Hirschhorn syndrome (WHS) patients have been comparatively evaluated for their clinical phenotypes and their respective genotypes. In eleven patients a 4 pter deletion, with proximal breakpoint varying from p15.1 to p16.1, was detected by conventional chromosome analysis. In five patients a microdeletion, whose size varied from 4.4 to 2.8 Mb, was detected by molecular probes only. In two unrelated patients the microdeletion was associated with a large 4p duplication. The last two patients had no deletions in an interval of about 500 kb spanning the WHS critical region (WHSCR). We found that genotype-phenotype correlations in WHS mostly depend on the extent of the deletion. We identified three clinical categories. 1)Patients harboring a large deletion usually presented with a severe phenotype, including markedly delayed psychomotor and physical growth, microcephaly (11/11), congenital heart defects (9/11), midline defects (9/11), renal abnormalities (5/11) and seizures(10/11). 2)Patients harboring a microdeletion of 4.4-3.2 Mb presented with a milder phenotype, usually lacking congenital malformations, with less severe developmental delay, but microcephaly still present. 3) The patient harboring the smallest microdeletion (2.8 Mb) and both nondeleted patients had a very mild expression of the disease: the head circumference was normal, congenital malformations were absent, mental and growth retardation was of a mild degree. If we look at WHS as a contiguous genes syndrome, the clinical manifestations observed in the nondeleted patients are likely to represent the "basic" phenotype of this condition, consisting of typical facial appearance, mild mental retardation, slight congenital hypotonia and mild growth retardation. Nondeleted patients presenting with a "basic" phenotype are extremely important in a general strategy for the identification of the gene(s) responsible for the WHS.

Program Nr: 2064 from the 1999 ASHG Annual Meeting

The efficiency of prenatal diagnosing of congenital malformations (CMs)in Belarus. *G.I. Lazjuk.* Dept Monitoring for Cong Malfo, Inst Hereditary Disease, Minsk, Republic of Belarus.

Under conditions existing in Belarus, congenital and inherited anomalies appeared to be the main cause of infants'diseases, disabilities and deaths. The main emphasis in prevention of the births of abnormal children in Belarus is laid on timely prenatal diagnosing(PD)with subsequent termination of pregnancy with incurable anomalies found in a fetus. Prenatal diagnosing is provided at 4 levels of medical care, at Maternal Counsellings(MC),Regional Medical Genetic Centers (RMGC), Belarus Medical Genetic Center(BMGC)and Belarus Institute for Hereditary Diseases. MC provide counsellings for 10,000 to 30,000 women. There, the blood is taken for prenatal biochemical screening and ultrasound screening of fetal condition is performed. RMGCs are available for the regions with the population of 1.3-1.5 million people. At RMGCs total ultrasound diagnosing of fetal condition and biochemical prenatal screening of the 2 nd trimester of pregnancy are performed. BMGC provides service for the residents of Minsk-city and Minsk region (3.3 million people) and all the patients, sent by RMGCs because they need more specific genetic examination. Various medical genetic examinations, practiced in Belarus, are provided at BMGC. Using all PD examinations in 1997 the anomalies were found in 512 fetuses, in 1998 the anomalies were recorded in 532 fetuses, that is 24.7% and 22.5% of all congenital malformations, registered by National Register, respectively. Of these anomalies, in 73% of cases congenital malformations were recognized before 22 weeks of gestation. Of PD methods, ultrasound diagnosing is found the most efficient at the populational level. Using ultrasonography more than 80% of all prenatally diagnosed CMs were revealed in Belarus. Invasive methods account for 23% of primary or confirming diagnoses. In 80% of prenatally diagnosed CMs, the women have their pregnancies terminated. When the anomaly is recognized after 22 weeks of gestation, only 50% of pregnancies are terminated. Practically all pregnancies with prenatally diagnosed chromosomal diseases are terminated. Each year about 480 pregnancies are terminated in Belarus because of diagnosed congenital anomalies.

Program Nr: 2065 from the 1999 ASHG Annual Meeting

Effects of BPA and OP on protamine-2 and P450 family gene expression of spermatogenesis in mice. *H.J. Lee[1], M.K. Kim[2], D.S. Ko[2], S.W. Han[2], D.H. Kim[2], H.G. Kang[2].* 1) Dept. of Physiology, Eulji Medical Science Instutite, Eulji Medical College, Seoul, Korea; 2) Eulji Medical Science Institute, Eulji Medical College, Seoul, Korea.

Recently, there are reported several factors, such as hormone, cytokines, transcription factors, others, which is affected on spermatogenesis. This study was done to investigate effects of endocrine disruptors (Eds) like as bisphenol-A (BPA) and 4-tert-Octyphenol (OP) on gene expression of spermatogenesis and to clarify the aromatase changes in testicular cells according to stages of testis when testicular cells were cultured with BPA and OP. Spermatogenic cells of mice were cultured for 3 days treated with 0.001-10 uM of BPA, OP and E2 as control group. To detect the gene expression of spermatogenesis in each group, gene expression of LDH-C4, p450 family were demonstrated by amplification of spermatogenic cells RNA by RT-PCR. This results shows that BPA and OP (0.1-10 uM) inhibited expression of protamine-2, CYP17 in spermatogenic cell for 3 day culture and also, expression of protamine-2 was inhibited in control group (E2). That is, BPA and OP were observed similar to functions like estrogenic effects on spermatogenesis. However, LDH-C4 were not inhibited in spermatogenesis and CYP11A1 which catalyses the first step in steroid hormone biosynthesis were expressed in all groups. As a result, normal spermiogenesis was inhibited in groups of E2 and BPA and OP treatment. In conclusion, it is suggested that phenolics Eds like as BPA and OP could have inhibitory effects of sperm maturation, especially spermiogenesis which is premeiotic process, in spermatogenesis.

Program Nr: 2066 from the 1999 ASHG Annual Meeting

Extensive survey of unexpected short limbs detected by fetal ultrasound screening.. *M. Le Merrer[1], M. Roubin[1], J. Amiel[1], P. Sonigo[2], N. Gigarel[1], V. Cormier Daire[1], P. Maroteaux[1], A. Muunich[1]*. 1) Genetique INSERM U 393, Hopital Necker, Paris, France; 2) Infantile radiology, hopital Necker, Paris, France.

The ultrasound measurement of femoral lenth is usually performed in France during the second and third trimesters of pregnancy. The aim of this study is to estimate itssensivity for detection of chondrodysplasias without familial history and its consequences for management of the pregnancy and delivery. From march 1996 to may 1999, a series of 149 pregnant women were refered for isolated short limbs (- 3 rd percentile) detected between 24th and 32rd weeks of pregnancy. In all cases fetal ultrasound measurements, in utero radiographies (performed around 28th weeks of pregnancy), fetal karyotype and FGFR3 mutation screening for achondroplasia were obtained and a tentative diagnosis with information on prognosis was given to parents in all cases. Post natal follow up revealed that : 1- A total of 42% of the newborns were normal with no features of chondrodysplasias regardless of antenatal growth 2- Non lethal chondrodysplasias were present in 45% of the newborns, essentially when prenatal short femora was noted during the second part of the pregnancy. Achondroplasia represented half of these cases (24%). Lethal chondrodysplasia was exceptional in our series as it was detected earlier than the 24 th week of pregnancy and interrupted . 3 - A total of 10% of newborns showed multiple malformations syndromes, not detected by ultrasound examination. 4- Finally, in 40 % of the cases, termination of pregnancy was decided by the informed parents according to the current legislation. The feasability and the reliability of the prenatal detection of chondrodysplasias by ultasound examination will be analysed . The possible factors associated to the decision to continue or terminate a pregnancy in which a chondrodysplasia has been diagnosed are discussed.

Program Nr: 2067 from the 1999 ASHG Annual Meeting

Prenatal diagnosis of X-linked ichthyosis by percutaneous umbilical blood sampling. *X. Li, H. Xiao, L. Li, G. Lu.*
Human Reprod Engineening Lab, Hunan Medical University, Changsha, Hunan, P. R. of China.

X-linked ichthyosis (XLI) is an inherited skin disorder caused by deficiency of steroid sulfatase (STS) activity and occurs in 1/2000-1/6000 males. The STS gene is located at Xp22.32. Over 90% of patients with XLI have large deletions of the STS gene. Prenatal diagnosis is considered to be an effective way to prevent the occurrence of this disease and has clinically been performed in this disease by FISH and metabolite or enzyme. We report a successful prenatal diagnosis of XLI by molecular genetic method. A 30-year old woman came to our clinic at the 27th week gestation for genetic counseling of XLI because she had previously delivered a male affected by XLI who was diagnosed by dermatologists according his typical ichthyotic skin. The boy except with XLI suffered from severe physical and mental dysgenesis and died of physical exhaustion at two years old. The pedigree showed six male relatives with XLI, but no one of six was with physical and mental dysgenesis. By percutaneous umbilical blood sampling (PUBS) we obtained fetal blood cells to prepare chromosome and genomic DNA. Chromosome analysis showed a karyotype 46,XY. PCR with primers for SRY confirmed that the fetus was male. In order to investigate for STS gene deletion we used two sets of primers (Ballabio A, Hum Genet 1990, 84:571) to examine the 5'- and 3'-regions of STS by means of PCR in several members of this family (the fetus, the pregnant woman , one of six relatives with XLI, who is the nephew of the pregnant woman and the patient's mother who is the pregnant woman's sister) and normal controls. The product of amplification of the 3'-region of STS in the pregnant woman's nephew wiht XLI was displayed by agarose gel electrophoresis, but no product of the 5'-region was seen. Two amplified products were seen in the fetus and all of others on agarose gel. Our result demonstrated that this male fetus had not deletion in 5'- or 3'-region of STS gene and chromosomal abnormality. The gestation was suggested to continue and a normal boy was born at the 40th week gestation. To our knowledge our report is the first case of prenatal diagnosis of XLI by PCR in the gene level.

Program Nr: 2068 from the 1999 ASHG Annual Meeting

DGAP: Developmental Genome Anatomy Project. *A.H. Ligon[1,5], G.A.P. Bruns[3,5], J.F. Gusella[2,5], B.R. Korf[3,5], R.L. Maas[4,5], M.E. MacDonald[2,5], A.M. Michelson[4,5], B.J. Quade[1,5], C.C. Morton[1,5].* 1) Depts. of OB/GYN & Pathology, Brigham & Women's Hospital, Boston, MA; 2) Dept. of Neurogenetics, Mass. General Hospital, Charlestown, MA; 3) Dept. of Pediatrics, Children's Hospital, Boston, MA; 4) Dept. of Medicine, Brigham & Women's Hospital, Boston, MA; 5) Harvard Medical School, Boston, MA.

DGAP has been established to identify genes critical in human development that are disrupted or dysregulated by balanced chromosomal rearrangements in patients with multiple congenital anomalies. These anomalies occur in approximately 3-4% of all livebirths, and can include clinically recognizable syndromes or isolated defects such as cleft palate/lip, limb defects, cardiac defects or mental retardation. Chromosomal abnormalities occur in 0.8% of liveborns, with ~50% showing an abnormal phenotype. Numerous structural chromosomal abnormalities have been reported as *de novo* in children with congenital developmental defects; a significant number of the breaks may disrupt or dysregulate genes critical to specific molecular pathways. We are collecting samples from patients with congenital anomalies and apparently balanced chromosomal rearrangements to use the breakpoints as signposts to locate critical genes. The potential of DGAP is enhanced by rapidly evolving genomic resources such as the complete human DNA sequence and an ordered FISH BAC map of the human genome. Collaborations between cytogeneticists and clinical geneticists have been formed to collect patient samples that reflect a variety of developmental defects. FISH analysis of chromosomal breakpoints will identify genomic clones containing candidate sequences. Molecular analysis of candidate genes will be the focus of subsequent studies, followed by expression analyses and isolation of orthologs in mice and *Drosophila*. Ultimately, transgenic and knockout animals will be used to study specific clones and to elucidate their roles in development. DGAP is a multi-laboratory, multi-institutional approach drawing on clinical genetics, cytogenetics, molecular biology and developmental genetics to illuminate genes involved in fundamental pathways of human development.

Program Nr: 2069 from the 1999 ASHG Annual Meeting

Comparison of Prenatal Ultrasound for Fetal Anomalies and Pathological Findings. *C. Llanusa, M. Gutierrez, J. Oliva, R. Sanchez, L. Carrillo, A. Nodarse, L. Rodriguez, I. Fundora.* Genetic, Gonzalez Coro Hospital, Havana City, Cuba.

The National Program for the Diagnosis and Prevention of Genetic Disease began in Cuba in 1984. This program includes the prenatal diagnosis of fetal malformations by maternal serum AFP screening at 15-19 weeks and fetal ultrasound at 22-24 weeks. Abnormal findings are followed by genetic counseling and the option of voluntary termination of the pregnancy. Gonzalez Coro and Clodomira Acosta hospitals are two neighboring maternity hospitals in Havana City where fetal ultrasounds are performed as part of the program. Between 1992-1996 the pathology department examined 220 malformed fetuses, stillbirths and/or neonatal deaths. Of these, 186 were pregnancy terminations because of abnormal ultrasound findings. One case was a stillbirth with anencephaly that had been diagnosed by ultrasound but opted to continue the pregnancy. The remaining 33 were cases in which ultrasound had failed to detect fetal anomalies, and the outcome was spontaneous stillbirth or neonatal death. The most common pathology diagnosis in the group of pregnancy terminations were neural tube defects (47), hydrocephalus and other CNS malformations (24), heart defects (24), ventral wall defects (18), renal anomalies (14), fetal edema (9) and MCA (7). The pathology diagnosis in this group coincided with the gestational ultrasound, although the former diagnosed additional anomalies not previously detected in 5 of the 7 cases of MCA. In the group of 33 malformed stillbirths and /or neonatal deaths in which ultrasound had failed to detect anomalies, the most common malformations found on pathology examination were MCA (7), renal anomalies (4), pulmonary hypoplasia (4) and heart defects (3). Overall, ultrasound detected 85% of anomalies. The proportion of correct prenatal ultrasound diagnosis by category was: neural tube defects 95.9%, ventral wall defects 94.7%, hydrocephalus other CNS malformations 88.8%, heart defects 88.8%, renal anomalies 77.7% and MCA 50%.

Program Nr: 2070 from the 1999 ASHG Annual Meeting

Elevated maternal serum alphafetoprotein in VACTERL-Hydrocephalus. *K.M. MacDonald[1,2], B.N. Chodirker[1,2], J.A. Evans[1,2]*. 1) University of Manitoba, Winnipeg, MB; 2) Children's Hospital, Winnipeg, MB, Canada.

Genetic counseling after a diagnosis of fetal hydrocephalus is problematic given the heterogeneous nature of the defect and the uncertainty of the prognosis. We report a male infant in whom hydrocephalus was identified during investigation of elevated MSAFP and who was found at birth to have VACTERL-H.

The mother of the proband was a 27-year-old G1P0 Canadian Aboriginal woman with well controlled Type II diabetes whose MSAFP at 19 weeks gestation was 2.6 mom. Fetal assessment revealed a disproportionately large head (75th centile) compared to femur length and abdominal circumference (both 5th centile) and lack of intracranial landmarks other than the falx. Amniotic fluid analysis showed elevated AFAFP (2.6 mom) but was gel negative for acetylcholinesterase. Karyotype was 46,XY. Follow up evaluations indicated increasing head size with only a thin rim of cortical mantle. Amniotic fluid volume remained normal. The infant was born at 35 weeks weighing 2840g (75th-90th centile) and with a head circumference of 46.9cm (>>95th centile). Additional anomalies suggesting VACTERL-H were cervical and sacral vertebral anomalies, tracheoesophageal fistula with esophageal atresia, renal dysplasia, thin distally placed thumbs, microphthalmia and micrognathia. MRI showed aqueductal stenosis. No increase in spontaneous or induced chromosome breakage was demonstrated on repeat chromosome analysis.

Although hydrocephalus has been observed with otherwise unexplained elevations of MSAFP (Burton, 1988), levels in pregnancies with VACTERL-H have rarely been reported. However, two infants in our series of cases with esophageal atresia (Chodirker et. al., 1994) had this diagnosis. Both had MSAFP levels at 1.6 mom and neither had polyhydramnios. Thus the mechanism of above normal AFP in this group may relate more to esophageal atresia than hydrocephalus. The diagnosis of VACTERL-H should be considered especially when apparently isolated fetal hydrocephalus is detected in women with high MSAFP, given that the diagnosis of esophageal atresia in the absence of polyhydramnios is difficult.

Program Nr: 2071 from the 1999 ASHG Annual Meeting

Nuchal Translucency Measurement in Twin Pregnancy. *J.N. Macri[1], D.A. Krantz[1], T.W. Hallahan[1], J.W. Larsen, Jr.[2], P.D. Buchanan[3], F. Orlandi[4], C. Rossi[4], L. Dugoff[5]*. 1) NTD Laboratories, Huntington Station, NY; 2) The George Washington Univ. Medical Center,Washington, D.C; 3) GeneCare Medical Genetics Center, Chapel Hill, N.C; 4) Centro Di Diagnosi Prenatale, Palermo Italy; 5) Univ. of Colorado, Denver Co.

Maternal factors have been used to adjust biochemical marker levels when screening for Down syndrome and open neural tube defects. To date, no maternal factors have been identified which effect nuchal translucency (NT) measurements. If the NT measurement of each fetus in a twin pregnancy tended to be similar, this might indicate that there are as yet unidentified maternal factors that effect NT values. A total of 32 first-trimester clinically normal twin pregnancies between gestational ages 10w4d and 13w6d in which NT was measured in both fetuses were analyzed. Using an analysis of variance procedure, data were analyzed to determine whether there was greater variation in NT measurements from woman-to-woman than between the twin fetuses in each pregnancy. Since NT increases with crown rump length data were analyzed using NT MoM results. The variance within the twin pairs was 42% of the variance among the twin pregnancies (.0651 vs. .1533). This was a significant difference (p=.009). To rule out operator variation as a cause of these results, a subset of 13 cases were analyzed in which the same operator performed all of the measurements. In this subset, the variance within the pairs was still less than the variance among the twin pregnancies (.0652 vs. .1065), however, this difference was not statistically significant. In conclusion, the data indicate that the 2 NT values in twin pregnancies tend to be associated although the association is not as strong when the measurement is performed by a single operator. This information suggests the possibility that maternal factors may affect the degree of nuchal translucency in the fetus.

Program Nr: 2072 from the 1999 ASHG Annual Meeting

ES Cell Neural Differentiation Reveals a Substantial Number of Novel Neural ESTs. *F.C. Mansergh, M.A. Wride, J. Hance, G. Bain, D.E. Rancourt.* Dept Biochem + Mol Biol, U. of Calgary, Calgary, Alberta, Canada.

We have developed methods for differentiating murine embryonic stem (ES) cells into functional neurons and glia in culture. ES-neural differentiation recapitulates the expression of neural markers in vivo and can generate large cell populations undergoing synchronous neural differentiation. We have therefore utilized this process to isolate novel genes expressed during early neurogenesis. Using a subtractive hybridization approach, we have isolated approximately 1200 cDNA clones expressed early in neural differentiation. Of 690 clones screened by single-pass sequencing, to date, 198 (27%) had no high level matches when analysed using BLAST and were therefore deemed novel. An additional 30 clones (4%) matched uncharacterized sequences. 131 (19%) matched genes that functioned predominantly in neural tissue, neural development or general developmental processes. 6% were involved in cell migration and proliferation, or cell cycle control. 24% represented housekeeping genes, while the remainder matched genes that were either incompletely characterized, or derived from non neural tissues. Pilot expression studies involving the first 10 clones identified 4 unique sequences that are expressed during neural development. RNase protection assays and whole mount in situ hybridization have revealed that 3 of these genes are preferentially expressed in the embryonic CNS. This system has the potential to identify genes which not only establish the embryonic nervous system, but also correlate with the over 800 chromosomal breakpoints and mapped or linked loci associated with congenital neurological handicaps and disorders in mankind. Given the recent discovery of human ES cells, this system also has great potential in neural drug discovery and cytotherapeutics.

Program Nr: 2073 from the 1999 ASHG Annual Meeting

Prenatally identified 47,XX,+8/47,XX,+14 karyotype. *J. Matheson[1], J. Santolaya[1], T. Nguyen[1], W. Jao[1], M. Freidine[1,2], M. McCorquodale[2]*. 1) Dept Ob/Gyn, Univ of Illinois, Chicago, IL; 2) Michael Reese Hospital, Chicago, IL.

A woman presented at 22 weeks gestation for evaluation of multiple fetal anomalies. Serial ultrasound evaluations revealed hypertelorism, dilated cisterna magna, micrognathia, cleft palate, VSD, ASD, omphalocele, bilateral renal pelvis dilatation, clenched fingers, clubfeet and increased amniotic fluid. Metaphase analysis of amniotic fluid cells revealed 47,XX,+8/47,XX,+14 mosaicism. At 42 weeks gestation, a 2600 g female infant was delivered and expired soon thereafter. In addition to previously identified anomalies, autopsy revealed low-set ears, absent gallbladder and double outlet right ventricle. Parental karyotypes were normal. Confirmatory FISH analyses were performed on different tissues.

Results of FISH studies in 100 cells/tissue for chroms 8 & 14

Tissue	Euploid	Tri 14	Tri 8	Tri8/Mono14
Blood	19	27	64	-
Skin	6	10	84	-
Placenta	20	64	10	6
Lung	9	3	66	20
Heart	31	65	3	-
Kidney	6	12	81	-

We propose the fetus was originally 46,XX and 2 separate cells underwent mitotic nondisjunction early in pregnancy leading to 3 independent cell lines. The differential survival of these cell lines may have improved the likelihood for viability until delivery.

Program Nr: 2074 from the 1999 ASHG Annual Meeting

Empirical validation of risk-based screening for Down Syndrome in Ontario. *C. Meier, AM. Summers, PR. Wyatt.* Genetics, North York General Hospital, Toronto, Ontario, Canada.

Since July 1993, triple marker maternal serum screening for Down syndrome in Ontario, Canada has been coordinated at the provincial level. As part of this coordination, all screening data is kept in a central repository, the Ontario Maternal Serum Screening Database (OMSSD), and is matched with pregnancy outcomes as they become available.

The database consists of the patient demographics, pregnancy details and results of over 400,000 screening tests. Triple marker screening involves combining a number of factors, including maternal age, race, diabetic status and the three biochemical markers (a fetoprotein, unconjugated estriol and chorionic gonadotrophin) to produce a single adjusted risk. Because of the relative complexity involved when computing these risks, the accuracy of the risk figures generated is not immediately obvious. One way in which the accuracy of the screening test can be determined is through **risk validation**, a process by which predicted risk is compared with actual disorder prevalence.

We used the OMSSD to categorize about 130,000 women who had received maternal serum screening in Ontario between October 1st, 1993 and September 30th, 1995 on the basis of their predicted risk for having a pregnancy affected by Down syndrome. We then compared the mean predicted risk in each risk category to the observed prevalence.

We found that the risk estimates generated closely matched the observed prevalence of Down syndrome, suggesting that the methodology used for screening here is accurate. We also evaluated the accuracy of other risk models.

Program Nr: 2075 from the 1999 ASHG Annual Meeting

Diagnostic dilemmas in prenatal identification of XX/XY mosaicism. *M.A. Micale[1], S.A.D. Ebrahim[2]*. 1) Dept Pathology, Medical Col Ohio, Toledo, OH; 2) Dept Pathology, Wayne State Univ, Detroit, MI.

Prenatal identification of a mosaic 46,XX/46,XY karyotype can be a counseling dilemma. Accepted practice for interpretation of such a karyotype, when ultrasound examination reveals a female fetus, would be that the XY cells might represent a sequestered or resorbed male twin. In the event that a male fetus is identified by ultrasound, the most likely explanation is maternal cell contamination (MCC). In addition, the presence of a chimeric fetal karyotype, while rare, cannot be excluded. We report two cases of sex chromosome mosaicism with unexpected outcomes, which demonstrate that the clinical significance of prenatally ascertained XX/XY mosaicism is not always straightforward. Case 1 is a woman referred for genetic counseling and amniocentesis because of multiple fetal anomalies. Cytogenetic examination demonstrated a 46,XX[14]/46,XY[6] karyotype while ultrasound revealed a fetus with female genitalia. Postnatal examination of cord blood and placenta revealed only XY with no evidence of XX cells in either tissue. Postmortem examination of the fetus revealed normal internal and external female genitalia. Thus the XX/XY fetal karyotype, originally believed to reflect a female fetus with a resorbed male twin, actually represented an XY female. Case 2 is a woman of advanced maternal age with a mosaic fetal karyotype 47,XY,+15[18]/46,XX[2]. The initial interpretation was a trisomy 15 male fetus, with the XX cells most likely representing MCC; however, ultrasound demonstrated a female fetus with no structural anomalies. A polymorphism on the short arm of chromosome 13 was shared in the XX and XY cells. Two percutaneous umbilical blood samplings (PUBS) revealed a 46,XX karyotype. Thus, the interpretation of these results in this ongoing pregnancy is a 46,XX fetus with a resorbed or sequestered male twin with trisomy 15. In addition, one of us (SADE) has previously reported a true chimeric XX/XY fetus. Based on these experiences, we suggest: 1) cautious interpretation of prenatally diagnosed XX/XY mosaicism even when ultrasound reveals the expected fetal sex and 2) confirmation of fetal sex by PUBS or postnatally by analysis of cord or peripheral blood.

Program Nr: 2076 from the 1999 ASHG Annual Meeting

b **Thalassemia mutation spectrum and prenatal diagnosis in Iranian population.** *H. Najmabadi[2], R. Karimi-nejad[1], S. Timorian[1], F. Pourfarzad[1], S. Homayounpour[1], A. Karimi-nejad[1], N. Lashkarian[1], N. Nabavi[1], T. Mohajerin[1], MH. Karimi-nejad[1]*. 1) Karimi-nejad Path & Genetic Center, Tehran, Iran; 2) Welfare & Rehabilitation University, Tehran, Iran.

Over 140 mutations have been determined for the b-globin gene. The mutation spectrum of bThalassemia in Iran is very heterogenous, and reflects east-west migration. Over a seven years period, we screened 873 individuals with bthalassemia minor and major for 20 common bglubin gene mutations by PCR ARMs analysis and nine RFLPs different systems were used. Because Iranians are a mixture of people from several Middle Eastern countries which have settled in different regions of Iran,we divided Iran into six regions to demonstrate the prevalence of different mutations in the North (N), Northwest (NW), Northeast (NE), Central (C), Southwest (SW), and Southeast (SE). A total of 313 families were screened,of which 33.4% were consanguineous marriages. Out of these consanguineously married couples,60.6% had the same mutation,31.2% were heterogenous for their mutations, and 8.2% were undetemined. Compared to the general population, homozygous couples were more common. Out of 179 amnion and CVs which were analyzed for prenatal diagnosis, 25.8% were affected, 44.5% had bthalassemia minor, and 23.3 % were normal for b globin gene. For 6.4% of the fetuses, we were only able to determine the condition of one chromosome. In conclusion we could detect only 70 to 85% of Iranian b thalassemia mutations and for the most of the remaining family RFLPs systems were informative. IVS II-I was the most common mutation in N, although it remained a common mutation throughout the country, it lessened in the east and IVS 1-5 became more common. As it is a prevalent mutation of Pakistan and India.

Program Nr: 2077 from the 1999 ASHG Annual Meeting

Mosaicism for trisomy 21 and sex chromosome monosomy (45,X/47,XX,+21) detected prenatally. *S.N. Palaniappan[1], A. Yearell[1], B. Bunke[1], M. Boschen[1], K. Kupke[2], K.M. May[1], D.F. Saxe[1].* 1) Div. Med. Genet., Dept of Peds., Emory Univ. Schl. Med., Atlanta, GA; 2) Neonatology Associates, Atlanta, GA.

Chromosome mosaicism in amniotic fluid can present difficult counseling issues concerning the fetal phenotype. We report a prenatal diagnosis of a double mosaic aneuploidy of 45,X/47,XX,+21, with a majority of the cells representing sex chromosome monosomy, presenting in a fetus with clinical features suggestive of Down syndrome. An amniocentesis was performed at 21.5 weeks gestation on a 32 year old woman referred for an abnormal multiple marker screen with an increased risk for Down syndrome. Initial FISH analysis on uncultured amniotic fluid cells revealed one signal for the X chromosome in 80% of the cells and a normal hybridization pattern for chromosome 13, 18 and 21, consistent with sex chromosome monosomy. Chromosome analysis of cultured amniotic cells revealed 47,XX,+21 (17%) and 45,X cells (83%), and no normal cells. Ultrasound did not reveal any fetal anomalies. The patient declined PUBS and elected termination. Examination of the fetus revealed low set and slightly posteriorly rotated ears, mid-face hypoplasia with slightly flattened nose and wide-set nipples. There was no evidence of webbing of the neck, or defects of the upper and lower extremities. The internal examination was unremarkable. Uterus, tubes and ovaries were present. Chromosome analysis of the skin, lung and kidney were unsuccessful due to cell growth failure. Analysis of the placenta from seven different sites revealed 45,X, 46,XX and 47,XX,+21 cell lines in various percentages. The presence of these cells lines could represent true mosaicism, a deceased co-twin or confined placental mosaicism. Molecular studies are pending.

True mosaicism of double aneuploidy is extremely rare, but has been reported for various aneuploidies. There has been one reported incidence of 45,X/47,XX,+21 prenatally diagnosed, in which the fetus presented with features of both Down and Turner syndrome. The present case emphasizes the need for documentation of clinical and cytogenetic findings for rare mosaic aneuploidies to aid in counseling regarding phenotype.

Program Nr: 2078 from the 1999 ASHG Annual Meeting

Chromosomal localisation and characterisation of *SOX18*, a developmentally expressed transcription factor. *D.J. Pennisi, G.E.O. Muscat, P.A. Koopman.* CMCB, University of Queensland, Brisbane, QLD, Australia.

The *SOX* gene family is an evolutionary conserved family of transcription factors that are developmentally expressed, with *SOX* genes involved in various aspects of development. Mutations in a number of *SOX* genes have been implicated in inherited disorders in humans and as the underlying cause of classical mouse mutations. We have cloned a member of this family, *Sox18*, and identify it as a gene involved in cardiovascular and follicle formation during development. We have mapped the human homologue to 20q13.3 by radiation hybrid mapping and it appears not to be associated with any inherited disorders. This allocation is consistent with the synteny between this region of the human genome and distal mouse chromosome 2 - the region where mouse *Sox18* has been mapped. We have produced mouse mutants null for *Sox18* and are currently conducting preliminary analyses.

Program Nr: 2079 from the 1999 ASHG Annual Meeting

Prenatal diagnosis of isolated femoral bent-bone skeletal dysplasia: Problems in differential diagnosis and genetic counseling. *P.G. Pryde[1], C. Zelop[2], R.M. Pauli[1]*. 1) Obstetrics and Gynecology, and Medical Genetics, U of Wisconsin, Madison, WI; 2) Obstetrics and Gynecology, U of Chicago, Chicago, IL.

Severe localized and symmetric bowing of the femora, in the absence of other nonskeletal abnormalities, is a rare prenatal ultrasound finding.

A 38-year-old woman was referred at 19 weeks gestation after triple marker screen estimated Down syndrome risk of 1 in 10. Family history was unremarkable. Ultrasound showed severe shortening and marked, symmetric bowing of the femora. The remainder of the long bones appeared normal in length, shape, and echodensity. Spine, calvarium, face, hands, feet, and thoracic skeleton appeared normal. Karyotype of amniocytes was 46,XY. A provisional diagnosis of kyphomelic dysplasia (KD) was provided. Despite the possibility of a favorable outcome, the patient elected termination of pregnancy by labor induction. Fetal post-mortem examination and radiographs were consistent with KD, although we feel that definitive distinction from femoral hypoplasia unusual facies syndrome (FH-UFS) may not, in this case, be possible.

KD is an autosomal recessive bent-bone dysplasia that, in contrast to campomelic dysplasia, involves principally the femora with relative sparing of the remainder of the skeleton. The diagnosis can be difficult to distinguish from symmetric cases of FH-UFS, which are thought in most instances to have sporatic inheritance or are seen in association with pregnancies complicated by diabetes mellitus. Although potentially lethal neonatal problems may arise, both KD and FH-UFS are compatible with probable normal life expectancy and normal neurodevelopment. The possibility that definitive distinction may not be possible prenatally, and even post-mortem (in cases choosing to terminate) is an important counseling consideration.

Program Nr: 2080 from the 1999 ASHG Annual Meeting

Cromosome prenatal diagnosis in Havana City, Cuba, 1984-1999. *J. Quintana, O. Quiñones, L.A. Méndez, M. Lavista, M. Gómez, N. Dieppa.* Dept Cytogenetics, Natl Ctr Medical Genetics, Havana, Cuba.

A National Program for the Diagnosis and Prevention of Genetic Diseases was implemented in Cuba in 1984. All components of the program are centrally planned and organized by the National Center of Medical Genetics and are financed by the governmental national health system free of charge. It includes newborn screening for PKU and congenital hypothyroidism, sickle-cell screening of pregnant women, maternal serum AFP screening, fetal ultrasound and prenatal diagnosis of sickle-cell and chromosome anomalies by amniocentesis (CVS was added in 1987). Indications for chromosome prenatal diagnosis are maternal age 38 years and over, positive family history, abnormal maternal serum screening and abnormal fetal ultrasound. Our laboratory provides centralized cytogenetic services for the western region of the country with a population of about 3 million, which includes Havana City and the provinces of Havana, Pinar del Rio and Isla de la Juventud. It also functions as a national reference laboratory for the rest of the country. Increased risk for chromosome anomalies in the offspring is detected early in pregnancy by family physicians at the primary care level following national criteria. Detected at-rik couples are referred to a network of genetic units where genetic counseling is provided and prenatal chromosome diagnosis is offered. Between January 1984 and April 1999, 6923 pregnant women underwent chromosome prenatal diagnosis (5138 by amniocentesis and 1785 by CVS). A total of 218 fetuses with chromosome abnormalities were diagnosed: 136 with aneuploidies (85 with trisomy 21), 41 with structural rearrangements (15 unbalanced) and 41 with mosaicisms. Abnormal results were informed to the couples by clinical geneticists following the principles of full and objective disclosure and non directive genetic counseling. The majority of couples with chromosomically abnormal fetuses requested to interrupt the pregnancy, a service also provided by the program. This included most cases with the milder sex chromosome aneuploidies.

Program Nr: 2081 from the 1999 ASHG Annual Meeting

Sources for misdiagnosis in preimplantation diagnosis of single gene disorders. *S. Rechitsky, O. Verlinsky, C. Strom, V. Ivakhnenko, J. Nefedova,, T. Amet, J. Pameranetz, M. Rechitsky, T. Kouliev, Y. Verlinsky.* Reproductive Genetics Inst, Illinois Masonic Medical Ctr, Chicago, IL.

Because preimplantation genetic diagnosis (PGD) of single gene disorders is based on single cell DNA analysis, its accuracy depends mainly on overcoming the major limitations of single cell PCR, which include allele drop out (ADO) and preferential amplification (PA). Both ADO and PA may be represented by a lack of one of the alleles in single heterozygous cell PCR analysis. With introduction of fluorescence PCR (F-PCR), there is a possibility to distinguish ADO from PA, allowing investigation of the proportion of PA detected as ADO by conventional methods. We tested 148 single fibroblasts heterozygous for cystic fibrosis Delta F-508 mutation by both conventional and F-PCR. ADO and PA rates were also recorded for Intron 6 polymorphism, which is closely linked to the gene. Our data showed that only a small proportion of lacking signals in conventional PCR were due to PA detected by F-PCR (1% for Delta F-508 mutation and 0.9% for Intron 6 polymorphism). This proportion is even lower following simultaneous amplification of both genes (0.6%). Our experiments also showed that the application of two markers picks up as much as 96.5% of all ADO, with almost no risk for misdiagnosis if 3 or more markers are included in multiplex PCR analysis. Our current experience includes PGD of 23 single gene disorders, performed by polar body biopsy and conventional or F-PCR. Overall, 66 clinical cycles has been performed by the present time, showing 97% diagnostic accuracy, based on the follow up analysis of 407 embryos resulting from oocytes predicted to have a mutant gene. The work has presently resulted in pre-selection and transfer of mutation free embryos in all but 3 cycles, with 23 established pregnancies resulting in the birth of 17 healthy children.

Program Nr: 2082 from the 1999 ASHG Annual Meeting

Combined elevation of maternal serum AFP and serum hCG predict adverse perinatal outcome. *S.B. Reddy, E. Ryu, S. Reddy.* AFP, National Medical Diagnostic La, Livonia, MI.

Elevated levels of maternal serum alphafeto-protein (MSAFP) in midtrimester are associated with neural tube defects, intrauterine growth retardation (IUGR) and other adverse pregnancy outcome. It has been demonstrated that elevated levels of serum human chorionic gonadotrophin (MShCG) during midtrimester are indicative of increased risks of IUGR and prematurity. The present report was designed to examine whether combined elevation of both MSAFP and MShCG lead to more pronounced pregnancy complications of adverse outcome than elevated MSAFP alone. Forty six patients with elevated MSAFP (>2.5 MOM) and MShCG (>3.2 MOM) and 64 patients with elevated MSAFP (2.5 MOM) as control group were studied in this report. In the present study, we observed that combined MSAFP and MShCG in midtrimester have significant increase in the rate of adverse preganncy outcome in 34 (73.9%) in contrast to elevated MSAFP alone in (1.6%). Stillbirth, fetal loss and neonatal death occured in 9 (26.5%) of the 34 patients with no losses among 64 control group. Major congenital defects were observed in 12 (35.3%) while none occured among controls. IUDR was noted in 13 (38.2%) compared to 1.6% among control group. These results indicate that a combined elevation of both MSAFP and MShCG in midtrimester is associated with a high risk of pregnancy outcome when compared to elevated MSAFP alone.

Program Nr: 2083 from the 1999 ASHG Annual Meeting

Pregnancy outcome for patients with an elevated first MSAFP sample and normal second sample. *J.R. Roberson, S. Diment.* Dept Medical Genetics, Henry Ford Hosp, Detroit, MI.

The Henry Ford Hospital MSAFP Program screening protocol allows a repeat serum sample for certain patients with an elevated initial sample (>2.2 MOM, <3.0 MOM and less than 19 weeks gestation). Patients who meet these criteria are offered ultrasound and/or amniocentesis if their second sample is screen positive. For those with a negative second sample, no further testing is offered. 155 patients with a negative second sample between 1995-1998 were studied to determine whether this protocol significantly reduced the false positive rate (FPR) and detection rate (DR)for open neural tube defects (NTDs) or had poor fetal outcomes in a higher frequency than expected.

24,549 initial serum samples were assayed between 1995-1998. 784 (3.2%) were elevated (>2.2 MOM). 521 (2.2%) were less than 3.0 MOMs and less than 19 weeks gestation,so were eleigible for a second sample. 182 of those had no second blood sample (first sample was recalculated based on ultrasound dating, declined a second sample, proceeded directly to amniocentesis or had early fetal demise). 339 (1.3%) of all patients had a second sample. 184 of those (54.3%) had a second elevation, while 155 (45.7%) had a negative second sample. 8 of 155 in the study group (5.2%) were lost to follow-up. One infant in this group had an open meningomyelocele and one had hypospadias. 112 were liveborn full term infants (38-42 weeks gestation) while 30 liveborns were born prematurely. Two of three fetal losses occurred before 24 weeks gestation. During this same screening period, 18 of 19 fetuses with neural tube defects were detected.

Conclusion: Although one fetus with NTD was missed because her second sample was < 2.2 MOM, our overal detection rate for NTDs during this period was 94% which is within the expected range. Allowing certain patients with an initial elevation to have a second sample reduced the FPR from 3.2% to 1.8%. Pateints with an initial elevated MSAFP who had a normal second sample still had a higher than expected rate of prematurity although they did not have a higher rate of fetal anomalies.

Program Nr: 2084 from the 1999 ASHG Annual Meeting

Safety of early amniocentesis: time to reevaluate CEMAT data. *R.M. Roberts.* Genetics and Prenatal Diag Ctr, Signal Mountain, TN.

The Canadian Early and Mid-trimester Amniocentesis Trial (CEMAT) found a significant excess of miscarriage, post-procedure leakage, and equinovarus malformation in the early (EA) group. As a result, the use of early amniocentesis has declined. Evaluation of the methodology of CEMAT can explain why their results are different from other studies, and in fact may reveal the cause of equinovarus--and how to avoid it. Here presented are the technical problems associated with EA. One of the most accessible pockets of fluid may be near the feet. At 10 to 14 weeks, the fetus has periodic episodes of a bicycling reflex, alternate repetitive flexion and extension of the legs. Fetuses who developed equinovarus may have been those who repetitively extended the feet onto a sharp needle tip, resulting in vascular disruption and tissue necrosis--hence a disruptive, not malformative etiology. This etiology of EA-associated equinovarus can be confirmed or rejected by reexamination of their videotaped procedures later resulting in equinovarus. Two other procedural factors may have contributed to their increased rate of miscarriage. The Canadian group had no instruction on what to do if the first aspiration resulted in no return of amniotic fluid. They can not know in how many such instances the operator re-aimed the needle tip laterally before attempting to re-aspirate. In many instances of no return, the amnion is tented, and not visualized by ultrasound. Lateral movement of the sharp bevel across the tense amnion could be the main cause of amnion leakage. I recommend never re-aiming the needle laterally without first withdrawing it to the uterine wall. The amnion is most easily punctured where it adheres to the chorion--hence, transplacental procedures should actually have less risk of post-procedure leakage, as their study found. Lastly, their assumption that removal of less amniotic fluid volume than usual would be safer could be wrong. I have found that the pressure in the extracoelomic space is 0, while inraamniotic fluid pressure can be twice that of mid-trimester pressure. Removal of a larger volume would result in lower intraamniotic fluid pressure, with less likelihood of the puncture site leaking prior to healing.

Program Nr: 2085 from the 1999 ASHG Annual Meeting

Prenatal diagnosis of duplication 4p mosaicism. *C. Rodriguez[1], T. Cimaroli[1], L. Sciorra[1], E. Guzman[2], J. Smulian[2], D. Day-Salvatore[1]*. 1) Division of Clinical Genetics, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ; 2) Maternal-Fetal Medicine, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ.

Duplication 4p syndrome is characterized by severe mental retardation, seizures, intrauterine growth restriction, clinodactyly, camptodactyly, and characteristic facial features; neither mosaicism nor prenatal diagnosis of this syndrome have been previously described. We report the prenatal diagnosis of two cases of *de novo* duplication 4p syndrome, one of which was mosaic, prompted by abnormal ultrasound findings. The first case was identified at 19 weeks gestation after amniocentesis was performed in a 32-year-old gravida 2 for a positive maternal serum triple screen for Down syndrome and 5th finger clinodactyly on ultrasound. The fetal karyotype was 46,XX,inv dup (4) (p12p16.3) in all cells analyzed. On autopsy, the fetus was noted to have clinodactyly, camptodactyly, preaxial polydactyly, horseshoe kidney, and characteristic facial features of duplication 4p syndrome. In the second case, a routine level II ultrasound at 19 weeks in a 31-year-old gravida 2 demonstrated bilateral absence of the middle phalanx of the 5th digit, single umbilical artery, echogenic focus in the left ventricle of the fetal heart, small fetal stomach, and one-week lag in fetal growth compared to the LMP. Amniocentesis revealed a karyotype of 46,XX[14]/46,XX,dup4(p16p16)[4] in the primary culture, with a mosaic result also identified in the subculture. This mosaic chromosome complement was confirmed in both a repeat amniocentesis and percutaneous umbilical blood sample. Postmortem examination could not be obtained. Chromosome analysis of both sets of parental bloods were normal. The prenatal diagnosis of structural chromosome abnormalities may be prompted by subtle, non-specific findings on ultrasound. In addition we report the first documented case of duplication 4p mosaicism.

Program Nr: 2086 from the 1999 ASHG Annual Meeting

Genomic cloning and characterization of the human homeobox gene *SIX9* reveals a cluster of *SIX* genes in chromosome 14 and associates *SIX9* hemizygosity with bilateral anophthalmia and pituitary anomalies. *S. Rodriguez de Cordoba[1], M.E. Gallardo[1], I. Fernaud-Espinosa[1], J. Lopez-Ríos[5], R. Sanz[2], C. Ramos[2], C. Ayuso[2], M.J. Seller[3], H.G. Brunner[4], P. Bovolenta[5]*. 1) Departamento Immunología, Centro de Investigaciones Biológicas (CSIC), Madrid, Spain; 2) Servicio de Genética, Fundación Jiménez Díaz, Madrid, Spain; 3) United Medical and Dental Schools, Guys Hospital, London, UK; 4) Department of Human Genetics, University Hospital Nijmegen, The Netherlands; 5) Departamento de Neurobiología del Desarrollo, Instituto Cajal (CSIC), Madrid, Spain.

The Drosophila gene *sine oculis* (*so*), a nuclear homeoprotein that is required for eye development, has several homologues in vertebrates (the *SIX* gene family). Among them, *SIX3* is considered to be the functional orthologue of so because it is strongly expressed in the developing eye. However, embryonic *SIX3* expression is not limited to the eye field and *SIX3* has been found to be mutated in some patients with holoprosencephaly type 2 (HPE2), suggesting that *SIX3* has a wide implication in head development. We report here the cloning and characterization of *SIX9*, a novel human *SIX* gene that is homologue of the chick *Six9*(*Optx2*) gene. *SIX9* is closely related to *SIX3* and is expressed in the developing and adult human retina. Data in chick and mouse suggest that human *SIX9* gene is also expressed in the hypothalamic and the pituitary regions. *SIX9* spans 2628 bp of genomic DNA and is split in two exons that are transcribed into a 1514 nucleotide-long mRNA. Chromosomal mapping of *SIX9* revealed that it is closely linked to *SIX1* and *SIX4* in human chromosome 14q22.3-q23, which provides clues about the origin and evolution of the vertebrate SIX family. Recently three independent reports have associated interstitial deletions at 14q22.3-q23 with bilateral anophthalmia and pituitary anomalies. Genomic analyses in one of these cases demonstrated *SIX9* hemizygosity, strongly suggesting that *SIX9* haploinsufficiency is responsible for these developmental disorders.

Program Nr: 2087 from the 1999 ASHG Annual Meeting

Role of Fetal ultrasound and molecular cytogenetics for karyotype-phenotype correlation of unbalanced structural chromosomal aberrations at prenatal diagnosis. *H.M. Ryu[1], M.Y. Kim[1], E.S. Kim[1], S.Y. Park[2], S.K. Choi[2], Y.H. Lee[3], H.W. Han[1].* 1) Dept OB/GYN, Samsung Cheil Hos, Sung Kyun Kwan Univ, Seoul, Korea; 2) Genetic Laboratory; 3) Dept Radiology.

The detection of unbalanced structural aberrations at prenatal diagnosis raises serious concern the phenotypic consequences on the fetus. The purpose of this study is to evaluate the role of the fetal ultrasound and molecular cytogenetics in predicting the prognosis of the fetuses with structural aberration. During July 1995 -June 1999, we performed about 3,781 fetal karyotyping, by which we detected 24 fetuses(0.63%) with unbalanced structural aberrations. There were 16 de novo cases and 5 familial cases and 3 unknown cases by parental karyotyping. For unbalanced structural chromosomal aberrations, we performed 1) detailed sonography and autopsy when the fetus was terminated or died in utero, 2) comparative genomic hybridization (CGH) and/or fluorescent in situ hybridization. Most common indication for fetal karyotyping was abnormality at fetal screening sonography. At detailed sonography, 18 fetuses showed abnormal findings; growth retardation in 6, oigohydramnios in 4, cardiac anomaly in 5, CNS anomaly in 6, and others. No abnormality was found at detailed sonography in 6 cases; three with structural aberration and three with marker chromosome. The cases with three structural aberration were terminated and showed no abnormality at autopsy. Two with marker chromosome showed normal CGH profiles and normal healthy babies were delivered. One with non-satellite marker chromosome was terminated. Seven cases where the conventional karyotyping was not conclusive, showed greater accuracy with CGH and/or FISH. Fetal sonography and molecular cytogenetics play an important role in predicting the prognosis of fetus with unbalanced structural aberrations at prenatal diagnosis.

Program Nr: 2088 from the 1999 ASHG Annual Meeting

Circumventing Male Infertility by ICSI should not Preclude Basic Evaluation. *S. Saitta, S. Zderic, G. Anadiotis, R. Colliton, L. Celle, R. Kline, N. Spinner, E. Zackai.* Children's Hosp of Phila, Philadelphia, PA.

Intracytoplasmic sperm injection (ICSI) was used for in vitro fertilization in a couple where the male had a decreased sperm count and low motility. Amniocentesis performed at 17 weeks showed a normal 46,XX karyotype in 15 cells studied. The pregnancy resulted in a newborn with abnormal genitalia including a shawl scrotum, single palpable gonad, and microphallus with hypospadias. Vaginal and uterine structures were found on a genitogram. Cytogenetic testing of the infant's peripheral blood lymphocytes revealed a mosaic karyotype of 46,XX[40]/47,XX,+r(Y)[10]. Surgical intervention included removal of both the scrotal gonad as well as the intraabdominal gonad due to increased risk of gonadoblastoma in this patient. Histology confirmed that the scrotal gonad was a testis, while the undescended gonad proved to be an ovotestis, providing evidence of true hermaphroditism. At this point, the father's peripheral blood karyotype was analyzed for the first time and showed a 46,Xr(Y) constitution in 100 cells studied. Highly polymorphic microsatellite DNA markers (heterozygosity greater than 70%) from the X chromosome (DXS990, DXS993, DXS999, DXS1001, DXS1227, PLP-CA, PLP-AhaII, PM2A) were used to analyze genomic DNA from the infant and father to determine the origin of the nondisjunction leading to the infant's 47,XX,+r(Y) lineage. Eight loci were studied and in all cases, the proband and father shared an allele, consistent with a paternal nondisjunction event resulting in this previously unreported mosaic karyotype. These findings illustrate the importance of routine karyotype analysis to help uncover the etiology of a patient's infertility, such as Y aneuploidy. This is underscored especially when newer technology-driven methods are used to circumvent the infertility.

Program Nr: 2089 from the 1999 ASHG Annual Meeting

Comparison of fetal cell recovery from maternal blood using high density gradient for initial separation step: 1.090 vs. 1.119. *O. Samura, A. Sekizawa, D.K. Zhen, V.M. Falco, D.W. Bianchi.* Division of Genetics, Dept. of Pediatrics, Tufts University School of Medicine, Boston, MA.

Objective: To improve the recovery of fetal nucleated erythrocytes (NRBCs) from maternal blood for noninvasive prenatal genetic diagnosis.

Methods: Samples were obtained from 27 women(28 samples)who had just undergone pregnancy termination at 6 to 23 weeks. Samples were split and mononuclear cells were isolated using Histopaque densities 1.090 and 1.119. CD45 depletion using magnetic activated cell sorting followed by flow-sorting with antibody to g globin and FISH analysis were used to evaluate the number of fetal cells recovered.

Results: In samples separated with the 1.119 density gradient, the yield of true anti-g positive cells (median: 14.9, range: 0-717.5)was significantly higher than that in the 1.090 density (median: 4.9, range: 0-532.5). In 13 out of 28 samples, the number of anti-g positive cells recovered from the 1.119 density gradient was two times or greater than that from the 1.090 gradient. After FISH analysis, in the 14 samples in which the fetal karyotype was different from mother, we detected a median range of 22.9 (range: 2-717.5) confirmed fetal NRBCs per 10-ml maternal blood in the 1.119 density gradient, whereas a median of 11.5 confirmed fetal NRBCs were detected in the 1.090 gradient (range: 0-532.5). The number of fetal NRBCs recovered from the 1.119 density gradient was significantly higher than from the 1.090 gradient.

Conclusions: Increased density results in improved fetal cell recovery in fresh post-termination maternal samples. The increased yield of fetal cells using the 1.119 density gradient may permit better noninvasive detection of fetal chromosome as well as DNA abnormalities in maternal blood.

Program Nr: 2090 from the 1999 ASHG Annual Meeting

MRI: An emerging tool in the assessment of fetal anomalies . *R.L. Schonberg, D.I. Bulas, K.D. Newman, K.N. Rosenbaum, C.J. Tifft.* Children's National Medical Center, Washington, DC.

High-resolution ultrasonography has been the traditional mode for the identification of fetal malformations. Magnetic resonance imaging (MRI) has further refined our ability to clarify the anatomy in some cases of suspected malformations. We report 2 cases where fetal MRI provided additional diagnostic information that influenced genetic counseling and pregnancy management.

Case 1 is a 36-year-old G2 P1 female whose fetus at 27 weeks gestation was suspected of having agenesis of the corpus callosum. The ultrasound was inconclusive due to fetal positioning. Images in multiple planes were obtained using a T2 weighted single slice technique with a Signa Horizon LX 1.5 Tesla GE scanner (Milwaukee, WI). Sedation was not used. The MRI established the the presence of the corpus callosum and the family was counseled that the risk of developmental delay was very small. The pregnancy continued to term with the birth of a healthy female infant.

Case 2 is a 29-year-old G1 P0 female who was evaluated by ultrasound at 21 weeks gestation. The fetus was found to have a large cystic adenomatoid malformation in the left lung resulting in severe mediastinal shift. No normal lung was visualized sonographically. MRI using the technique described above was performed at 23 weeks gestation and showed a large multicystic left upper lobe mass. Normal appearing lung was identified in the lower left lobe and right lung. The infant delivered at 39 weeks gestation. No immediate intervention was required.

The use of fetal MRI in our patients resulted in improved diagnostic accuracy and assessment of fetal outcome. This technique will continue to evolve as an important adjunct to ultrasound imaging in selected pregnancies.

Program Nr: 2091 from the 1999 ASHG Annual Meeting

Rescue of a conditional p53 mutation by the *lac*repressor in preimplantation mouse embryos. *H. Scrable, W. Gluba, L. Ligon, A. Leung.* Dept Neuroscience, Univ Virginia, Charlottesville, VA.

The *p44*gene encodes a truncated version of p53 that is missing the amino-terminal overlapping domains for transactivation and mdm2 binding. We have inserted *lac*operator sequences into the promoter region of *p44*downstream of the transcription start site. In assays of reporter function *in vitro*, the modified *p44*promoter was found to be fully regulatable by lactose when introduced into cultured cells along with the *lac*repressor. A line of transgenic mice carrying this conditional *p44*mutation was created on a *p53 (+/+)*background. *p44*expression paralleled that of *p53*in all tissues examined, but at a consistently higher level. Adult male *p44*transgenic mice developed giant cell degenerative syndrome of the testis, which has been associated with reduced levels of p53. *p44*transgenic mice were mated to a line of transgenic mice homozygous for the bacterial gene encoding the *lac*repressor. Adult male *p44, lacI/lacI*mice did not develop testicular giant cell degenerative syndrome. To determine what effect this had on gamete formation, we compared the viability of sperm from *p44*males to *p44, lacI/lacI*males by counting the number of one-cell embryos harvested from hormonally-prepared, non-transgenic females of a similar genetic background to which they had been mated. There was no difference in the fertilization rate of sperm derived from males of either genotype. When the embryos were cultured, however, many more embryos derived from the *p44*male died at the morula to blastocyst transition than did embryos derived from the *p44, lacI/lacI*male. The difference in embryo death rates was statistically very highly significant (*p=0.00088*). Taken together, these data suggest that p53 has both an effect on gamete formation in the testis and a male-derived, epigenetic effect on preimplantation embryogenesis that occurs at the time of blastulation, and that these effects are independent of each other.

Program Nr: 2092 from the 1999 ASHG Annual Meeting

Alpha fetoprotein (MSAFP) screening:Our five years experience. *M. Segovia, L. Lopez Miranda, L. Alba, M. Mollica, A. Rebechi, O. Pivetta.* Centro Nacional de Genetica Medica, Buenos Aires, Argentina.

The purpose of this research work is to present the outcomes of a group of patients with unusually high maternal serum a-fetoprotein levels. 3000 MSAFP determinations were performed in our center during 5 consecutive years, between 15 and 21 weeks of gestation. 75 (2.5%) of the 3000 samples showed high MSAFP levels. In 41 (54.6%) of the samples the results obtained were: 10(24.4%) fetal deaths with X=4.33MOM(dn=1.6;dn-1=1.68); 2(4.87%)presented anencephaly with X=8.55 MOM(dn=3.45;dn-1=4.80); 2(4.87%) gastroschisis with X=7.7MOM(dn=1.75;dn-1=2.47); 2(4.87%) omphalocele with X=3.7MOM(dn=0.1; dn-1=0.14); 2(4.87%)retroplacental haematomas with X=2.85 MOM ((dn=0.25;dn-1=0.35);1(2.4%) craniorachischisis with 23 MOM; 1(2.4%) risk of abortion with 5.9 MOM; 1(2,4%) oligohidramnios with 3.6 MOM; 1(2.4%) spontaneous abortion with 22.7 MOM; 1(2.4%) moderate hydronephrosis with 2.4 MOM; 1(2.4%) ascites plus complex heart defect with 3.0 MOM; 17(41.4%) twin pregnancies with one X=2.4 MOM(dn=1.0;dn-1=1.03). There is no apparent clinical reason for the remaining 34(45.3%),although the most probably cause could be: 1) inaccuracy to determine the gestacional age; 2)blood samples obtained a few days after chorionic villus sampling. In our research the most common findings with high MSAFP levels were fetal death (24.4%) and twin pregnancies (41.4%), and the highest MOM values were found in pregnancies with neural tube defects. In conclusion , the aim of this work is to emphasize that abnormally high alpha fetoprotein values, even with normal ultrasound findings, are a reliable sign to identify high risk pregnancies.

Program Nr: 2093 from the 1999 ASHG Annual Meeting

Outcome of pregnancies after maternal renal transplantation. *M. Sgro[1], T. Barozzino[1], T.M. Mirghani[2], M. Sermer[2], H. Akoury[2], D. Chitayat[3]*. 1) Deparment of Pediatrics, The University Health Network, University of Toronto, Toronto, ON, Canada; 2) Department of Obstet & Gynecology, The University Health Network, University of Toronto, Toronto, ON, Canada; 3) The Prenatal Diagnosis Program, The University of Toronto, Toronto, ON, Canada.

We studied retrospectively the outcome of pregnancies of women recipients of renal transplant, who were on cytotoxic medication prior to conception and for the duration of the pregnancy. There were 44 pregnancies in 30 women and 32 of them resulted in liveborn children. One child was lost for follow-up. Information regarding the growth and development was obtained on 31 children through direct assessment or telephone interviews. The mean maternal age was 28 years (19-38 years). The mean interval between the renal transplantation and conception was 5 years. Cytotoxic medications used included: cyclosporine + azathioprin + prednisone in 24, azathioprin + prednisone in 13 and cyclosporin + prednisone in 5. The average gestational age at delivery was 36 weeks and 44% (14) were delivered prior to 37 weeks. The average birth weight was 2.53 kg and 3 children had birth weight <3rd centile. One child (3%) was born with multiple congenital abnormalities. The range of follow-up was 3 months to 11 years (mean 3.1 years) and no additional congenital abnormalities were detected on follow-up. Diabetes mellitus type 1 developed in one child, moderate to severe sensorineural hearing loss in 1, 2 children had asthma and 1 child had recurrent otitis media. One child had a learning disability and one child had prevasive developmental delay. Conclusion: long term follow-up of children born to renal transplant recipient mothers does not show an increased incidence of congenital and/or developmental abnormalities or late onset conditions compared to the general population. There was an increased incidence of premature delivery and low birth weight.

Program Nr: 2094 from the 1999 ASHG Annual Meeting

Detecting prominent nuchal translucency among first-trimester fetuses with autosomal abnormalities: assessing frequency and operator experience. *L.P. Shulman[1, 2], O.P. Phillips[1], D.S. Emerson[1], Y. Tunca[1], A.T. Tharapel[1]*. 1) Univ of Tenn, Memphis; 2) Dept of Ob/Gyn, Univ of Ill at Chicago.

The detection of a prominent nuchal translucency (PNT) during the ultrasound exam of a first-trimester fetus is associated with marked increased risk for fetal aneuploidy. Indeed, PNT measurement is now an integral part of investigational first-trimester screening protocols. PNT screening is dependent on ultrasound equipment and operator experience; accordingly, current practice calls for special training and certification for those performing aneuploid risk assessment. Much has been written about the ability of PNT to detect fetal aneuploidy with relatively little reported about the frequency of PNT among fetuses with aneuploidy. We sought to assess the frequency of PNT among fetuses with autosomal aneuploidy and the role of ultrasound experience. All women presenting for first-trimester prenatal diagnosis were evaluated by a group of experienced sonographers who subsequently received certification, in early 1998, to provide aneuploidy screening. We prospectively studied all first-trimester fetal autosomal aneuploidy cases from 1994 to 1998. Scans were performed between 9.5 and 12.9 weeks; all were performed on the same ultrasound machines. PNT was defined as a separation of skin from body wall ³ 2.5 mm. Approximately 85% of women presented for prenatal diagnosis for AMA (³ 35 years at EDC). Results: Ninty-five consecutive cases were included in this study. In 1994, 38.5% of fetuses with autosomal aneuploidies were prospectively found to have PNT. This increased to 47.1% in 1995, 65% in 1996 and 76.2% in 1997. In 1998, 70.8% of the 24 affected fetuses had PNT. No other atypical ultrasound findings were reported. Conclusions: Although PNT will detect many fetuses with autosomal aneuploidy, approximately 30% of affected fetuses will have normal first-trimester scans. Operator experience plays a role in detecting PNT and could affect screening outcomes. Such information is thus critical for women with normal first-trimester scans. Uniform training and certification may serve to diminish the impact of operator inexperience and maintain the screening efficacy of PNT measurement.

Program Nr: 2095 from the 1999 ASHG Annual Meeting

Skewed X-inactivation in Incontinentia Pigmenti (IP2). *A. Smahi, S. Heuertz, J.P. Bonnefont, A. Delanay, P. Vabres, A. Munnich.* Genetique INSERM U393, Hopital Necker, Paris, France.

Incontinentia Pigmenti (IP2) or Bloch-Sulzberger syndrome is a rare neurocutaneous syndrome characterized by developmental abnormalities of the tissues and organs derived from the embryonic neuroectoderm. The mode of inheritance is X-linked, dominant, with male lethality. Highly skewed X-inactivation has been observed in peripheral blood leukocytes and fibroblasts of IP2 patients (Migeon B.R., et al., 1989; Harris A., et al., 1992; Parrish et al., 1996). We analyzed the pattern of X-inactivation in blood leukocytes of a cohort of 49 sporadic and 70 familial cases of IP2. A total of 98.5% of affected females showed a non-random X-inactivation. Otherwise, a random X-inactivation was observed at the vesicular stage of the disease in two one-year-old girls. These results suggested that the apparent skewed X-inactivation is the result of selection occurring in the early postnatal development against cells expressing the mutated IP2 gene on the active X-chromosome. The study of X-inactivation allowed us to determine the parental origin of the mutant allele in sporadic cases. In 71% of sporadic cases, the paternal X-chromosome was inactivated, suggesting that the IP2 mutation occurred in the father's germline. The remaining 29% were shown to inactivate their maternal X-chromosome. Gonadal and somatic mosaicism were also occasionally noticed. These data indicate that highly skewed X-inactivation is a hallmark for IP2 and is therefore of considerable help in establishing diagnosis in "mild" IP2 phenotype. Thus, combining the X-inactivation test and linkage analysis is required for accurate genetic counseling in IP2.

Program Nr: 2096 from the 1999 ASHG Annual Meeting

Ectopic expression of Sonic Hedgehog from the human Keratin 1 promoter gives rise to limb abnormalities and skin lesions in transgenic mice. *I. Smyth[1], J. Bowles[1], J. Rothnagel[2], C. Wicking[1], B. Wainwright[1]*. 1) CMCB, University of Queensland, Brisbane, Australia; 2) Dept of Biochemistry, University of Queensland, Brisbane, Australia.

The Patched/Sonic Hedgehog signalling pathway controls the differentiation of a range of tissues and organs including the central nervous system, the limbs and the skin. The involvement of this pathway in human disease has been demonstrated by the finding of mutations of Patched in the naevoid basal cell carcinoma syndrome (NBCCS), which is characterised by predisposition to basal cell carcinoma (BCC) and medulloblastoma, and by a variable range of developmental defects. Tumorigenesis in this syndrome is thought to result from homozygous inactivation of the Patched protein, which in turn leads to the constitutive activation of an associated protein, Smoothened. We have endeavoured to model this inactivation by over-expressing the hedgehog gene in transgenic mice, a process which also leads to constitutive activation of Smoothened. A full length rat sonic hedgehog cDNA was expressed from the human keratin 1 (HK1) promoter which is transcribed in mitotic interfollicular basal cells and in suprabasal layers from around 15 days post coitum. The resulting mice exhibit limb defects similar to the syndactyly occasionally noted in NBCCS patients. They also develop inguinal skin lesions which display sebaceous differentiation and unusual pigmentation. Immunohistochemistry indicates that these lesions express the BCC markers keratin 6 and keratin 14, but do not express proteins characteristic of suprabasal epidermal layers, such a loricrin. Transgenic back skin is phenotypically normal, however tail and foot skin exhibits basal cells hyperproliferation and abnormal dermal pigmentation. These studies demonstrate the ability of ectopic Sonic Hedgehog to alter epidermal development, even when expressed at a late embryonic stage, and also suggest that tumour like lesions can form as a result of expression of the transgene in interfollicular basal cells.

Program Nr: 2097 from the 1999 ASHG Annual Meeting

A structural mutation of *HOXA1* may be associated with autism spectrum disorders (ASDs). *C.J. Stodgell[1], D.A. Figlewicz[2], S.L. Hyman[3], P.M. Rodier[1]*. 1) Obstetrics and Gynecology, Univ. Rochester Sch. Med., Rochester, NY; 2) Neurology; 3) Pediatrics.

ASDs are common congenital diabilities, occurring in 2-6/1000 births. Several lines of evidence suggest that some cases involve very early injury resulting in craniofacial anomalies, maldevelopment of the brain stem, and cranial nerve dysfunctions, along with the behaviors diagnostic of autism. Null mutation of *Hoxa1* in mice leads to a similar set of somatic and neurologic deficits. While examing the sequence of the *HOXA1* gene in humans cases, we found a region of the 2nd exon that could not be amplified by PCR. When the primers were moved distally, the sequence was amplified successfully, and proved to match the published sequence, suggesting that the defect in the 2nd exon is structural, and is generated by a change in sequence outside of this exon. Semi-quantitative ratings of 51 probands and 112 controls indicated that the structural anomaly is strongly associated with ASDs. Its pattern of inheritance is unusual. Some parents homozygous for the wild-type allele have 2 children with the structural variant. This suggests genetic anticipation. These characteristics argue that the variant is important, and prompted a search for the source of the structure. We used a restriction endonuclease (Pvu II) to digest genomic DNA from a proband homozygous for the variant. The large fragment containing the *HOXA1* coding region was retrieved by size-selecting 3-5kb fragments from an agarose gel, cloning them, and testing the excised inserts to find ones that contained the 1st and 2nd exons of *HOXA1*. Surprisingly, the sequence of a clone positive for *HOXA1* revealed the presence of *HOXA2*, a gene that is known to be over 8kb away. Further, 5 Pvu II cut sites should be present between the coding regions of the 2 genes. This suggests that a deletion of about 3,500 bases is present, eliminating the cut sites and brings the genes closer together. Further description of the deletion in probands and controls will tell us whether the deletion is unstable, as the inheritance pattern suggests, and will allow a definitive quantification of the distribution of the variant in probands and controls.

Program Nr: 2098 from the 1999 ASHG Annual Meeting

Evaluation of prenatal diagnosis of congenital anomalies by fetal ultrasonographic examination in Europe. *C. Stoll[1], M. Clementi[2]*. 1) Service de Genetique Medicale, Hopital de Hautepierre,Strasbourg, France; 2) Registry of congenital anomalies for the prenatal U.S.Diagnosis Working Group, Padova, Italy.

Ultrasound scans in the midtrimester of pregnancy are now a routine part of antenatal care in most European countries. Thanks to registries of congenital anomalies a study was undertaken in Europe. The objective of the study was to evaluate prenatal detection of congenital anomalies by routine ultrasonographic examination of the fetus. All congenital malformations suspected prenatally and all congenital malformations, including chromosome anomalies, confirmed at birth were identified from the Congenital Malformation Registers, including 21 registers from the following European countries : Austria, Croatia, Denmark, France, Germany, Italy, Lithuania, Spain, Switzerland, The Netherlands, UK, Ukrainia. These registries are following the same methodology. The study was performed between july 1996 and december 1997, including 407.000 pregnancies per year. 5041 babies were judged abnormal at delivery. The percentage of detection was variable for the diverse categories of congenital anomalies; it was high for neural tube defect (NTD) 94.5 per cent for anencephaly, 73.3 per cent for spina bifida, but low for ventricular septal defect and for atrial septal defect (ASD), 9.2 per cent and 4.9 per cent, respectively. However the detection rate was higher for multiply malformed children (for example 4 times higher for ASD). Detection rate varied between European countries. Termination pregnancy rate was high for central nervous system anomalies (52.9 per cent) and chromosomal anomalies (49.8 per cent) and low for renal anomalies (24.3 per cent) and congenital heart defects (14 per cent). Overall 24.5 per cent of all pregnancies were terminated after prenatal detection of congenital anomalies. This study showed that many fetuses with major malformations can be identified prenatally in routine practice. Because policies, methods and techniques continually change, ongoing surveillance of prenatal diagnostic services is vital.

Program Nr: 2099 from the 1999 ASHG Annual Meeting

Fraser syndrome: Prenatal Diagnosis and autopsy findings. *M. Thomas[1], A. Toi[2], W. Sirkin[3], H.A. Gardner[4], L. Moore[5], D. Chitayat[1]*. 1) The Prenatal Diagnosis Program,The University Health Network, University of Toronto; 2) Department of Diagnostic imaging,The University Health Network, University of Toronto; 3) The Department of Pathology, North York General Hospital, Toronto,ON, Canada; 4) Genetic Services, Lakeridge Health Centre, Oshawa,ON,Canada; 5) Dept. of Radiology, Lakeridge Health Centre, Oshawa,ON,Canada.

Fraser syndrome is an autosomal recessive condition with multiple abnormalities including cryptophthalmos, syndactyly, genital, nose, ear, laryngeal, renal, and skeletal abnormalities, cleft lip and palate, umbilical hernia and mental retardation. Death is usually associated with laryngeal atresia or renal agenesis. We report the prenatal ultrasound and autopsy findings on a case with Fraser syndrome. The mother was a 28-year-old G1P0 and the father was 30 years. The couple was healthy and distantly related. Detailed fetal ultrasound at 21 weeks gestation revealed mild cerebral ventriculomegaly, thick nuchal fold, echogenic and large lungs with inverted diaphragm and cardiac compression, absent lift kidney and malrotated right kidney. There was ascites, abnormal hands, low umbilical cord insertion and abnormal genitalia. The findings were felt to be consistent with Fraser syndrome. The pregnancy was terminated and the autopsy confirmed the ultrasound findings. The facial features were dysmorphic with short palpebral fissures and eyelids coloboma, notched alae nasi, microstomia and micrognathia. The ears were low set and posteriorly rotated, the nuchal fold was thick, the chest was distended and the abdomen was prominent. There was a low insertion of the umbilical cord, the external genitalia was abnormal and there was syndactyly of the fingers and toes. Internal examination revealed bifid uvula, tracheal stenosis with cricoid cartilagenous ring, distended lungs with abnormal lobation, left renal agenesis and malrotated right kidney. The fetal karyotype was normal (46,XY). The prenatal U/S finding of enlarged and echogenic lungs in association with ascites should raise the possibility of laryngeal atresia and Fraser syndrome should be included in the differential diagnosis of such cases.

Program Nr: 2100 from the 1999 ASHG Annual Meeting

Evaluation of Downs Risks obtained from a Triple Screening Program in African-Americans. *T.F. Thurmon, K. Yanamandra, D. Napper, H. Chen, S. Ursin.* Dept Pediatrics-Genetics, Louisiana State Univ, Shreveport, LA.

LSU Medical School began a triple analyte (MSAFP, MSHCG and MSuE3) screening program serving pregnant women from Northwest Louisiana, a few years ago. To evaluate our program and to improve the quality of screening, we started analyzing the MSAFP elevations generating NTD risks and the Down syndrome risks generated for the outcome of chromosomal defects.

Typically we see higher Downs risks in African-Americans (AA) over European-Americans (EA). We make adjustments to the MSAFP MOM for weight, race and diabetes status, however we only make weight adjustment to HCG and uE3. Unconfirmed LMP dating revealed much higher risk differences. Thus we eliminated this variable by comparing risks only through US dating. We also refined the comparisons by excluding twins, diabetes status, and AMA. However, the risks were still higher in AA even after these exclusions.

After excluding several variables, we found that the predominant marker contributing to the higher Downs risks in AA community was the MSHCG. MSAFP and MSuE3, were still contributing to the difference but not as much. Even after excluding AMA cases, age difference could still be a factor. So, we compared the maternal ages between the two races but found minimal difference. The mean and median maternal ages in our program in the non-AMA category in AA and EA were 22.4, 23.1, 21, and 22 years respectively. Although it is easy to conceptualize separate MSAFP, MSHCG and MSuE3 medians for each race, it may not be practical in smaller programs and with cumbersome software development for each race especially if the patient population was small. Also, it cannot be discounted of the importance of using local population medians lest false risks can be generated.Thus in our view making race adjustment to MSHCG MOM and possibly to MSuE3(race adjustment to MSAFP is likely in practice in most prenatal centers)may be a practical alternative in eliminating some of the problems in the risk calculations for individual races. Data and statistics will be presented.

Program Nr: 2101 from the 1999 ASHG Annual Meeting

The First Trimester Multiparametric Screening is to Become the Standard of Prenatal Diagnosis. *G.L. Tsukerman[1, 2], I.A. Kirillova[1], N.B. Gusina[1], S.A. Schreder[1], L.M. Lishtvan[1], S.I. Kovalev[1], G.A. Krapiva[1], L.A. Savenko[1], O.V. Pribushenya[1], L.V. Podleshuk[1], E.Y. Jaroshevitch[1], L.K. Shamgina[1]*. 1) Center for Med Genetics Serv, Inst Hereditary Diseases, Minsk, Republic of Belarus; 2) Reproductive Genetics Institute, Chicago, IL, USA.

Before introduction of the whole population multiparametric first trimester prenatal screening ultrasound examination with special attention to nuchal translucency (NT)of 27,265 first trimester pregnant women with parallel biochemical testing of 15,000 serum samples, including more than 400 samples at various fetal anomalies, for AFP, free b-hCG and partly for PAPP-A was carried out for 3 years (1996-1998). 481 empty sacs and non-viable fetuses, 215 multiple pregnancies, and 67 cases of fetal malformations including 48 neural tube defects were revealed. NT³3 mm. was noted in 633 of the fetuses. Karyotyping of 417 of them gave us the possibility to detect 34 cases of aneuploidies (trisomy 21-12 cases, trisomy 18-7 cases, trisomy 13-3 cases, monosomy X-11 cases, and 1 case of triploidy). 37 cases of Down's syndrome were screened in the first trimester. NT measurement was performed in 29 of them, 12 fetuses had NT³3 mm. and 2 cases more could be detected using the risk calculation program. The Gaussian distribution parameters were estimated for Down's syndrome and unaffected pregnancies and used for risk calculation. At cut-off risk ³ 1:360 at the expected date of delivery detection rates for different markers combinations were: 77% for all three, 73% for PAPP-A and free b-hCG and 65% for AFP and free b-hCG (6; 7 and 8% false-positive rate, respectively). A modelling exercise for the screened population with NT, AFP, PAPP-A and free b-hCG predicts a 92% detection rate for Down's syndrome at 6% false-positive rate. It is still surprising that the first trimester screening has not become the standard of prenatal care yet.

Program Nr: 2102 from the 1999 ASHG Annual Meeting

A New Technique of Depositing Blastomeres onto Polycarbonate Filters for FISH. *W.D. Weber[1], C.H. Lytle[2], L.L. Estabrooks[2], K.T. Rapiejko[1], M.A. Lee[3], E. Johnson[3], G. Miller[1].* 1) Research & Develoment, Genzyme Genetics, Framingham, MA; 2) Genzyme Genetics Santa Fe, NM; 3) Fertility Center of New England Reading, MA.

Preimplantation Genetic Diagnosis(PGD) is a powerful technology capable of generating additional genetic information through the isolation and analysis of individual blastomeres from a developing embryo. Problems in the developmental process of the fetus can be predicted through specific genetic changes such as chromosomal aneuploidy, deletions or mutations. Molecular analysis of single blastomeres can be carried out through PCR amplification techniques, however information regarding individual chromosomes are lost since PCR is not quantitative.

Fluorescence In Situ Hybridization(FISH) can identify chromosomal aneuploidy that can be associated with fetal loss. Detection of chromosomal aneuploidy would potentially increase the success of IVF while reducing fetal loss, stillbirths and live children born with multiple anomalies. Advances in FISH technology have enabled multiple targets to be tested simultaneously in interphase nuclei providing specific information about chromosomal number in less than 4 hours. Currently FISH is performed on a blastomere after the deposition onto a microscope slide, a process that is technique dependent and difficult to control.(J. Harper et al., 1994)

We have identified a deposition methodology where the blastomeres are applied to polycarbonate filters for subsequent FISH analysis. This eliminates many of the problems associated with the current slide based deposition. An overview of this method will be presented.

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Program Nr: 2103 from the 1999 ASHG Annual Meeting

Prenatal Diagnosis, Cytogenetic analysis and Postnatal FISH study of a structurally abnormal Y chromosome in a patient with Turner syndrome. *J.L Weiss[1], K.P. Magee[1], S.W. Cheung[2]*. 1) OB/GYN, Methodist Hospital, Dallas, TX; 2) Laboratories for Genetic Services, Inc. Houston, TX.

It has been suggested in the literature that all live born females with Turner syndrome carry a cell line containing two sex chromosomes, which may be present at low level of mosaicism. Delhanty JD et al (Ann Hum Genet 1998 62:99-106) reported that 2 of the 50 patients with a 45,X karyotype by conventional cytogenetic analysis were positive for six of the eight Y chromosome loci using PCR. Additional cytogenetic analysis confirmed the presence of a rearranged Y chromosome in 8% and 3% of the cells respectively. We reported a case of a 21-year-old primigravida with an abnormal triple screen suggesting an increased risk for Down syndrome (1 in 92). Amniocentesis was performed at 20 weeks gestation with level II ultrasound examination. Gender appeared to be female. The initial chromosome analysis from amniocytes revealed a 45,X chromosome pattern thereby indicating a fetus affected with Turner syndrome. This analysis excludes 20% mosaicism with 99% confidence when 20 colonies of cells are analyzed from 3 independent cultures. The patient elected to continue the pregnancy. Upon delivery, male genitalia was identified in the 6 lb. 4.4 oz infant and cord blood chromosome analysis revealed a 45,X[14]/46,X,idic(Y)(q11.2)[16] chromosome pattern. We retrospectively analyzed all the metaphase cells available on the amniotic culture slides and found 3.5 % of the colonies to have the idic (Y) (i.e. one colony each from the 3 primary cultures). Physical examination revealed a male infant with stage I hypospadias. A MRI revealed a 1.8 x 2.0-cm mostly fluid filled structure posterior to the bladder possibly representing an uterine remnant. A single testicle was identified on the right side of the scrotum. Neither testicle nor ovary was identified on the left side. Congenital heart disease was suspected and confirmed by an echocardiogram revealing an atrio-septal defect and a patent ductus arteriosus for which no intervention was required. The infant physically did well and was discharged on day three. Detailed cytogenetic studies and clinical observations of the infant will be presented.

Program Nr: 2104 from the 1999 ASHG Annual Meeting

Evaluation of maternal serum triple screen as identifier of trisomic fetuses. *S.L. Wenger, J. Lane, L.R. Brancazio, G.R. Hobbs, Jr.* West Virginia Univ, Morgantown, WV.

Maternal serum triple screen identifies women under 35 years of age who are at risk for carrying a fetus with Down syndrome. In order to detect 60% of trisomy 21 fetuses, the screen is designed to identify 5% of all women tested to be at increased risk. Of these women that undergo follow-up amniocentesis, 2-3% have a trisomy fetus. To determine our rate of detection, we retrospectively looked at amniocentesis performed for reasons of maternal serum triple screen or advanced maternal age since 1993. Medical records for approximately 800 cases in each category were reviewed for woman's age, risk of Down syndrome, karyotype results and number of ultrasound abnormalities. Our results showed that among 1600 women, six trisomy 21 and three trisomy 18 (all advanced maternal age) fetuses were identified. Among the maternal serum screen group 0.38% were trisomic, and among the maternal age group 0.75% were trisomic. While 5% of all ultrasounds had one or more abnormalities, they were present in 67% of trisomic fetuses. Currently less than half of pregnant women who are counseled about their increased risk for a fetus with Down syndrome have an amniocentesis. Our low detection rate may reflect that either not enough amnios are being performed to detect at risk pregnancies, or that our population is different from that of the national norm.

Program Nr: 2105 from the 1999 ASHG Annual Meeting

Challenges in the prenatal diagnosis of Walker-Warburg syndrome. *P.G. Wheeler[1], D. Levine[2], T.L. Stewart[1], D.W. Bianchi[1]*. 1) Division of Genetics, Department of Pediatrics, Tufts University School of Medicine, Boston, MA; 2) Dept. of Radiology,Beth Israel Deaconess Medical Center, Boston, MA.

Walker-Warburg syndrome is an autosomal recessive condition involving anomalies of the brain(lissencephaly and other structural defects), eye(cataracts and anterior chamber defects) and muscular dystrophy. We have recently had a case that highlights the difficulties and importance of making this diagnosis.

Abnormalities in the 1st pregnancy of a 33-year-old woman were first detected at 33 weeks' gestation when a prenatal ultrasound examination was performed for maternal concerns of decreased fetal movement. It showed marked hydrocephalus, cerebellar hypoplasia, and possible club feet. Fetal head MRI showed severe hydrocephalus,porencephaly,a non-dilated 4th ventricle, and cerebellar hypoplasia. The corpus callosum was not visualized. The preliminary diagnosis was cerebro-cerebellar dysplasia with porencephaly. Delivery occurred at 34 weeks. At birth, multiple joint contractures and minimal muscle mass were noted. The neonate died at 1 day of age from respiratory failure. At autopsy, partial lissencephaly, hydrocephalus and absence of the corpus callosum were detected. A muscle biopsy done shortly after death found abnormalities consistent with a congenital muscular dystrophy. Examination of the eyes revealed early sub-capsular cataracts. These findings are consistent with the diagnosis of Walker-Warburg syndrome.

The diagnosis of Walker-Warburg syndrome in the baby highlights several important factors, including that this diagnosis typically can not be made based on fetal ultrasound or fetal head MRI alone, since neither study generally detects the lissencephaly seen in this condition. Also, without a complete autopsy (including muscle pathology and eye dissection) the diagnosis of Walker-Warburg syndrome cannot be made accurately when it is the first presentation in the family. The accurate diagnosis of this condition is essential since as an autosomal recessive condition it has a 25% recurrence risk, which is considerably different than the neglible recurrence risk for porencephaly.

Program Nr: 2106 from the 1999 ASHG Annual Meeting

***SOX14* maps to human chromosome 3q22-23 and is expressed in the central nervous system and apical ectodermal ridge of the developing chick limb.** *H.P. Wilmore, M.J. Smith, S. Wilcox, K.M. Bell, A.H. Sinclair.* Dept of Paediatrics, Centre for Hormone Research, University of Melbourne and Murdoch Institute, Royal Childrens Hospital, Melbourne, Australia.

Members of the *SOX* gene family encode proteins with homology to the HMG box DNA-binding domain of *SRY*, the Y-linked testis-determining gene. A number of *SOX* genes have been shown to be important for normal embryonic development. Mutations in *SRY*, *SOX9* and *SOX10* are responsible for XY sex reversal, campomelic dysplasia and Waardenburg-Hirschsprung disease, respectively. In order to identify novel human *SOX* genes, a genomic library and two foetal brain cDNA libraries were probed with PCR products obtained using degenerate primers for known *SOX* (HMG) box sequences. One of the positive clones represented the human *SOX14* gene and the sequence of the entire coding region was obtained. Homology between the amino acid sequences places *SOX14* within the subgroup of *SOX* genes containing *SOX1*, *SOX2*, *SOX3* and *SOX21*, all of which are expressed in the central nervous system. In order to investigate the embryonic expression pattern of *SOX14*, we used PCR to amplify the chick orthologue and this was used as a probe in whole mount *in situ* hybridisation analysis of day 2 to day 6 embryos. Expression was seen in the ventral intermediate zone of the neural tube and in the apical ectodermal ridge (AER) of the developing limb. This is the only SOX gene known to be expressed in the AER, a structure which directs outgrowth of the embryonic limb bud.

The expression patterns and assignment of genes to human chromosomes has previously been used to correlate *SOX* genes with human disease phenotypes and known chromosomal locations. We therefore carried out FISH analysis, which localised *SOX14* to human chromosome 3q22-23, and further localised the gene to a 3Mb YAC within this region. Two human congenital syndromes which have been mapped to this chromosomal region are BPES (blepharophimosis, epicanthus inversus and ptosis) and Moebius syndrome. *SOX14* is therefore a candidate gene for these syndromes.

Program Nr: 2107 from the 1999 ASHG Annual Meeting

In Utero Fetal Therapy Using Pig Tail Shunts (1990-1998). *R.D. Wilson, M. Bebbington, D. Farquharson, F. Galerneau, N. Kent, D. Money, K. Williams, F. Tessier, A. Gagnon, K. Lim.* Division of Maternal-Fetal Medicine, University of British Columbia, Children's & Women's Health Center, Vancouver, British Columbia, Canada.

OBJECTIVE: In-utero fetal therapy using pig tail shunts has been a therapeutic option for a number of years. The most common anatomical areas for which this therapy is recommended are bladder, thorax and abdomen. This present report will review the in-utero fetal therapy pig tail shunting experience at the University of British Columbia, B.C. Women's Hospital from 1990-1998.

METHOD: This retrospective review evaluated cases identified through the fetal treatment register at B.C. Women's Hospital, Centre for Prenatal Diagnosis and Treatment. All fetuses that underwent evaluation whether or not a shunting procedure was undertaken were included. Complete outcome is available for this population including morbidity and mortality.

RESULTS: For bladder obstruction 19 pregnancies were worked up for obstructive uropathy. 10 had poor renal function while 6 had good renal function with 3 undergoing serial bladder aspirations and 3 undergoing vesicoamniotic shunting. Three did not have any evaluation of renal function. For the thorax, 12 pregnancies underwent work-up for unilateral/bilateral fetal pleural effusions. Three cases underwent thoracoamniotic shunting. Nine had serial aspirations only. Abdominal pathology was present in 7 cases with 1 case undergoing peritoneal-amniotic shunting. Other cases were treated with single or serial aspirations.

CONCLUSIONS: The appropriate selection of cases for in-utero shunting is required. Fetal morbidity and mortality is high within this treatment group and often shunting procedures do not improve fetal outcome in anatomical areas other than bladder neck obstruction.**OBJECTIVE**.

Program Nr: 2108 from the 1999 ASHG Annual Meeting

Congenital apnea in newborn mice lacking the Na^+/myo-Inositol cotransporter (SLC5A3) gene. *S. WU, R. BUCCAFUSCA, J. GOLDEN, J.J. MALLEE, A.D. LUCENTE, K.E. McVEIGH, G.T. BERRY.* Departments of Pediatrics and Pathology, The University of Pennsylvania School of Medicine, The Children's Hospital of Philadelphia, Philadelphia, PA.

The known roles of myo-Inositol (mI) are to serve as precursor of the phosphoinositides, important in signal transduction, and as an intracellular osmolyte. A millimolar cellular concentration gradient for mI is maintained by Na^+-dependent mI transport in certain brain, peripheral nerve, ocular and renal cells. The only gene identified to date whose product is capable of active mI transport is SLC5A3 and its transcription is under osmoregulatory control. As part of a study designed to analyze SLC5A3 function *in vivo*, the murine homologue of SLC5A3 was cloned. Similar to human tissues, its transcription was found to be high in adult murine tissues such as brain and kidney. It is also expressed in murine mid- and late-gestational stage embryos and in placenta. Consistent with the abscence of active mI transport, no expression of SLC5A3 was found in striated muscle tissues, and minimal in liver. To evaluate the developmental and physiological role of SLC5A3-mediated high affinity, Na^+-dependent mI transport, mice lacking SLC5A3 (SLC5A3 -/-) because of targeted gene inactivation were generated. At postnatal 14 days of age, of 170 offspring of (SLC5A3 +/-) X (SLC5A3 +/-) matings, 63 were SLC5A3 +/+, 107 were +/- and 0 were -/-. The SLC5A3 -/- animals die shortly after birth without establishing sustained respiration. Initial anatomical and histological examinations of these mice at birth have not revealed gross abnormalities. These results suggest that the SLC5A3 gene is not critical for embryonic development, *de novo* mI synthesis by the fetal-placental unit probably being sufficient, as well as responsible for the relatively high concentrations of mI reported in fetal blood. The respiratory failure in SLC5A3 (-/-) newborn pups is probably due to restricted, albeit lethal, post-natal deficits in cellular mI availability. This model that simulates the stillborn may provide insight into the molecular mechanisms of control of respiratory/cardiovascular function and pathogenesis of apnea in human infants.

Program Nr: 2109 from the 1999 ASHG Annual Meeting

The Role of M6p/Igf2r in Mouse Heart Development. *A.A. Wylie[1,3], D.J. Pulford[1,3], J.G. Falls[1], P.H. McDonald[2], T.C. Orton[3], R.L. Jirtle[1].* 1) Radiation Oncology, Duke University, Durham, NC; 2) Departments of Medicine (Cardiology) and Biochemistry, Duke University, Durham, NC; 3) Safety of Medicines Department, Astra-Zeneca Pharmaceuticals, Cheshire, UK.

The M6p/Igf2r is a cell membrane associated glycoprotein involved in lysosomal targeting, degradation of the mitogen Igf2, and activation of the mitoinhibitor TGFb. The M6p/Igf2r has been mapped to the mouse Tme locus and is genomically imprinted with exclusive expression from the maternal allele. Several lines of evidence support the involvement of M6p/Igf2r in embryonic growth and organogenesis. M6p/Igf2r knockout studies in mice demonstrated that maternal inheritance of a M6p/Igf2r null allele as well as homozygosity for the inactive allele was lethal at birth. Histological analysis of these mice revealed multisystem defects and in particular abnormalities of the heart and lungs. Hearts were 2-5 times larger than controls and were characterized by a disorganized ventricular myocardium. To further address the role of the M6p/Igf2r in embryogenesis we have generated transgenic mice in which the single functional allele of M6p/Igf2r can be conditionally inactivated using the CRE-loxP recombination system. In particular we investigated the contribution of developmental defects in the heart to the lethal phenotype of M6p/Igf2r knockout mice. A transgenic line expressing CRE-recombinase under the control of the cardiac specific MLC-2v promoter was crossed with a transgenic line in which exon 10 of M6p/Igf2r was flanked by two loxP sites. CRE expression in the hearts of F1 hybrids results in the excision of exon 10, generating a M6p/Igf2r gene that encodes for a truncated protein lacking the entire extracellular domain of the receptor. Our data indicates that loss of M6p/Igf2r in the heart during development by itself is not lethal and the mice appear phenotypically normal. Using the same CRE-loxP system, we are now investigating the contribution of defects in lung development to the lethal phenotype of M6p/Igf2r knockout mice.

Program Nr: 2110 from the 1999 ASHG Annual Meeting

Functional and Structural Dissection of a novel Human BETA3 Gene, a bHLH Transcription Factor. *Z. Xu[1], A. Dutra[2], J. Piatigorsky[1]*. 1) Laboratory of Mol.& Dev. Biol., National Eye Institute, Bethesda, MD; 2) National Human Genome Research Institute Bethesda, MD20852.

The genes encoding basic helix-loop-helix proteins have been implicated in many aspects of neural development, including cell growth, differentiation and migration of precusor cells and postmitotic cells. A neural specific bHLH protein, human BETA3 (hBETA3) was cloned by homologous screening of human BAC library and subsequently mapped to chromosome 8q13. The mouse homologue gene (mBETA3) was mapped to chromosome 3qA3, which is a region of conserved synteny with human chromosome 8q13. Several candidate genes have been mapped to chromosome 8q13 in human, including Duanes syndrome type II. Genomic sequence analysis of the human and mouse BETA3 gene indicate that it contains a single exon, which is about 3.75 kb encoding a 381 amino acids bHLH protein (37 kDa predicted molecular weight). Multiple alignments of amino acid sequences of known BETA3 genes indicates several conserved motifs, and an identical 147 amino acid C-terminal region which contains a 60 amino acid bHLH domain. A 27-bp trinucleotide repeats (CAG)9 encoding poly-serine was found in human BETA3, and only one CAG in the corresponding position of mouse and hamster BETA3.Northern analysis of human BETA3 gene revealed its brain-specific expression pattern, which is most abundant in cerebellum. A putative transcription factor which contains two bHLH domains was identified by searching Caenorhabditis eleganss database, and each of the bHLH domains showed 73% and 63% identity to human BETA3 bHLH domain.

Program Nr: 2111 from the 1999 ASHG Annual Meeting

A MOUSE MODEL FOR RIEGER SYNDROME. *B. Yang[1], E. Semina[2], T. Hjalt[2], X. Cao[1], R.S. Reiter[3], P.A. Kirby[4], R.F. Hrstka[1], J.J. Lin[3], R.A. Williamson[1], J.C. Murray[2,3].* 1) Dept OB/GYN, Univ Iowa, Iowa City, IA; 2) Dept Pediatrics, Univ Iowa, Iowa City, IA; 3) Dept Biologocal Science, Univ Iowa, Iowa City, IA; 4) Dept Pathology, Univ Iowa, Iowa City, IA.

Rieger syndrome is an autosomal dominant human disorder affecting eye, tooth and umbilical development, and whose primary morbidity is the 50% occurrence of glaucoma in affected individuals. The identification of etiologic mutations in the PITX2 gene and its encoded protein, solurshin, inspired the development of a transgenic animal that might serve as a model for this disorder. In addition, the more recent identification that PITX2 also plays a role in left-right axis determination also suggests that both heterozygote and homozygote knockouts might contribute to our understanding of cardiac development, as well. The mouse PITX2 homolog was identified and inactivated through gene targeting by deleting the homeobox region of the gene. Mice heterozygous for the deletion show Rieger-like in the craniofacial features, including smaller teeth and eyes, increased height of the skull, and smaller length of the mandible compared with their wild-type littermates. The heterozygotes are otherwise normal, including body weight, size, and fertility of both sexes. Mice homozygous for the Pitx2 deletion die in utero around embryonic day 9.5, though our analysis thus far does not suggest the cause of death. The heart, placenta, and umbilical core are normal and development is not obviously impaired. Further analysis of the homozygous embryos is ongoing.

Program Nr: 2112 from the 1999 ASHG Annual Meeting

X-inactivation patterns in cytotrophoblast vs tissues of human female fetuses. *S. Zeng[1], J. Yankowitz[1], J.C. Murray[2]*. 1) Dept OB/GYN, Univ Iowa Hosps & Clinics, Iowa City, IA; 2) Dept Ped, Univ Iowa Hosp & Clinics, Iowa City, IA.

One of the two X-chromosomes is inactivated in the early embryogenesis of female mammals to compensate for gene dosage compared to males. In the embryonic tissues the inactivation is random. There is controversy concerning human extra-embryonic tissues. Some have hypothesized and offered limited data to support preferential paternal X-inactivation. Some of the studies have not delineated the nature of the placental tissues studied, and there may be differences based on whether one evaluated the villus core versus the cytotrophoblast. We hypothesized that there would be skewed X-inactivation in human cytotrophoblast.

Placental tissue was collected from 137 first trimester abortuses and a variety of fetal tissues (heart, lung, liver, kidney, muscle, intestine, brain) were obtained when possible. Cytotrophoblasts were isolated by established protocols involving enzymatic digestion, filtration, Percoll density gradient and monoclonal antibody bound to magnetic beads. Sixty-three were females as identified by use of PCR for an X-Y marker. X-inactivation was analyzed using methylation status of the first exon of the androgen receptor gene. Of the 55 heterozygotes 37 displayed random inactivation (skewing 50-65%) and 18 had skewed inactivation (mild skewing >65-75%, n=9, moderate >75-85%, n=8, and severe >85%, n=1). Tissues were obtained from 24 of the 55 female fetuses (heart n=20, lung n=16, liver n=19, kidney n=23, muscle n=24, intestine n=24, and brain n=19: not all tissue types could be obtained from each fetus), only two instances of skewed inactivation were seen. This was in the liver (67.8%) and intestine (75.4%) of different fetuses. Our results indicate a greater degree of skewing in the extraembryonic tissues compared to embryonic tissues. However, it would appear that preferential X-inactivation in the placenta is not absolutely required for normal human embryonic or extra-embryonic development. The etiology of the skewing and the developmental implications are not clear.

Program Nr: 2113 from the 1999 ASHG Annual Meeting

A novel Pax-6 binding site with a high similarity to a region in B1 repetitive elements. *Y.-H Zhou[1], J.B. Zheng[1], X. Gu[2], W.-H Li[3], G.F. Saunders[1].* 1) Department of Biochemistry and Molecular Biology, The University of Texas M. D. Anderson Cancer Center, Houston, TX; 2) Department of Zoology-Genetics, and Iowa Computational Biology Laboratory (ICBL), Iowa State University, Ames, IA; 3) Department of Ecology and Evolution, University of Chicago, Chicago, IL.

Pax-6 encodes a paired domain and homeodomain transcription factor that plays an important role in the development of eye and central nervous systems. Through various combinations of its DNA binding domains, Pax-6 achieves specific and modular transcription regulation from their multiple DNA binding domains. Since the Pax-6 consensus binding sequence is relatively long, it is applicable to screen Pax-6 binding sequences from genomic DNA or search them from sequence database. Using an in vitro approach for the isolation of PAX6 binding sequences from a *Mbo* I digested human single-copy genomic DNA pool, we have identified a novel PAX6-specific binding site hGCa1BLs1 which has a high similarity with the 29-bp duplication region in B1 repetitive element. We examined the corresponding sequences in the consensus B1 elements for PAX6 binding by electrophoretic mobility shift assays (EMSA). As expected, PAX6 does not bind to consensus B1. However, mutation of G to T in position 93 or A to T in position 103 in the consensus B1 sequences examined leads to a gain of PAX6 binding ability. Search of database for B1 elements that contain the putative active PAX6 binding sequences retrieved 34 unique sequences from the rodent genome and one from the primate genome. Some are in functional genes which will be asigned for functional analyses. Sequence alignment analysis reveals that 14 sequences exhibit the modern B1 feature, while most of the other sequences exhibit feature of proto-B1 elements PB1 or PBD7. Phylogenetic analysis showed that these putative Pax-6 binding B1 sequences were mixed with rodent null B1 sequences included in the tree. All these analyses suggests that the Pax-6 binding B1 elements arose before the divergence of the primate and the rodent, and the null B1 could be re-activated by a single-step mutation. Thus B1 repetitive elements have the potential to be involved in the evolution of gene regulation by pax-6.

Program Nr: 2114 from the 1999 ASHG Annual Meeting

Exclusion of Neuropilin-2 as a candidate gene for Familial Primary Pulmonary Hypertension (FPPH). *K.B. Lane[1], R. Gaddipati[1], N. Fretwell[2], W.C. Nichols[3], FPPH Consortium[1,2,3,4].* 1) Department of Medicine, Vanderbilt University, Nashville, TN; 2) Dept. of Genetics, Leicester University, University Road, Leicester; 3) Division of Human Genetics, Children's Hospital Medical Center, Cincinnati, OH; 4) Indiana University School of Medicine Indianapolis, IN.

Primary pulmonary hypertension (PPH) is a vasocclusive disease of the pulmonary circulation. Prior to vasodilation therapy the average survival from diagnosis to death was 3.5 yrs. Six percent of PPH patients have an affected relative. Transmission is autosomal dominant with reduced penetrance. While the ratio of affected females to males is 2:1, the mortality curves for the sexes are similar. Father-to-son transmission is noted in several families. Five generations of transmission have been detected. In subsequent generations the disease is detected at earlier ages, displaying genetic anticipation, which has been linked to tri-nucleotide repeat expansions (TRE). Previous linkage analysis by us has localized the FPPH gene to a 10 Mb region of human genome at 2q32-q33 with a LOD score of >7. Cloning and EST makers have identified »100 transcription units in this region. We have begun screening candidate genes found in the region. A vascular endothelial growth factor (VEGF) receptor gene, neuropilin-2 (Nrp-2), was identified near the telomeric end of the FPPH CI. VEGF is involved in neovascularization, endothelial and SM growth, and recanalization, all associated with the lesions seen in PPH. A receptor for VEGF was considered a high priority candidate gene. EST primers for Nrp-2 were obtained and Nrp-2 expression in the lung was confirmed by RT-PCR. A 3' cDNA was obtained by PCR amplification. This probe was used to screen northern blots from control, FPPH, PPH, and Interstitial Pulmonary Fibrosis (IPF) lung RNA. No apparent differences, in quantity or size of the transcript were noted. Southern hybridization did not detect any alterations in genomic DNA structure associated with Nrp-2. Importantly, recombination at several novel microsatellite markers near and within Nrp-2, including one shown to be centromeric to NRP-2 by physical mapping, was detected which excludes NRP-2 and all genes telomeric as the FPPH gene.

Program Nr: 2115 from the 1999 ASHG Annual Meeting

Disruption of the dystrophin binding site of b-**dystroglycan in the mouse.** *J.C. Lee[1], M.D. Henry[1], R.F. Hrstka[2], R.A. Williamson[2], K.P. Campbell[1]*. 1) Departments of Physiology and Biophysics and Neurology, HHMI, University of Iowa College of Medicine, Iowa City, IA; 2) Department of Obstetrics and Gynecology, University of Iowa College of Medicine, Iowa City, IA.

The dystroglycan complex is composed of two proteins which are transcribed from a single gene and post translationally modified to produce a-dystroglycan, a 156 kD extracellular protein and b-dystroglycan, a 43 kD transmembrane protein. Based on its wide tissue distribution, the dystroglycan complex is believed to play diverse roles in various tissues. In skeletal muscle, the dystroglycan complex is part of the larger dystrophin-glycoprotein complex and contributes to the overall structure and function of the cell by creating a linkage between the extracellular matrix and the intracellular cytoskeleton through interactions of a-dystroglycan with laminin and b-dystroglycan with dystrophin. Dystroglycan also binds with high affinity to Dp116 in peripheral nerve. It is important in basement membrane formation, epithelial morphogenesis and as a receptor for certain viruses. Previous studies have determined that the 15 C-terminal amino acids of b-dystroglycan contain the binding site for both dystrophin and Dp116. In order to further elucidate the role of b-dystroglycan in skeletal muscle and other tissues, we introduced a premature stop codon eliminating the dystrophin binding site in murine embryonic stem (ES) cells through targeted homologous recombination. Biochemical analysis of total cell homogenates from heterozygous ES cells indicated that both the native (43 kD) and truncated (41.5 kD) forms of the protein were produced. *In vitro* analysis of ES cells homozygous for this mutation will allow us to examine the consequence of this mutation on the organization of the intracellular cytoskeleton. In addition, there may be effects of this mutation on a-dystroglycan and its laminin binding properties. Mice heterozygous for the truncated form of b-dystroglycan were viable and appeared healthy. Analysis of mice homozygous for the truncation is currently underway. Given the diverse roles of dystroglycan, it will be interesting to see the effect of this mutation in muscle and other tissues in the mouse.

Program Nr: 2116 from the 1999 ASHG Annual Meeting

Differential Expression of Human Genes in Hippocampus and Cerebellum. *S-J. Lee, L.E. Varnavas, C.B. Naves, R.J. Zahorchak.* R&D Departmant, Research Genetics, Huntsville, AL.

The hippocampus is responsible for learning and memory of intellectual functions and cerebellum for motor movement. The relative expression levels of genes in human hippocampus and cerebellum were determined using Human Gene Filters (Research Genetics Inc.) containing 25,000 genes hybridized with probes prepared from mRNAs from pooled human hippocampi and cerebella. By searching the differential expressed genes, we want to explore the candidate genes responsible for the differential functions between hippocampus and cerebellum. Collected data analyzed by Pathway 2.01 (Research Genetics Inc.) were calculated as relative ratios of gene expression in hippocampus to that in cerebellum. The obtained results indicated that 43 genes were expressed more abundant in hippocampus than in cerebellum by a factor of two or greater and that 16 genes were expressed more abundant in cerebellum than in hippocampus by a factor of two or greater. Myelin basic protein (MBP) was the gene most highly differentially expressed in hippocampus compared to cerebellum. The gene expression of MBP, which was expressed 3-4 fold higher in hippocampus compared to cerebellum, was confirmed by an independent method using Human RNA Master Blot (Clontech, Inc.) probed by MBP cDNA sequence. The later analysis also showed that MBP mRNA was highly and specifically expressed in 14 human brain tissues when compared to 28 other body tissues. Among brain tissues, MBP mRNA was expressed the least in cerebellum and the most in spinal cord. The protein expression level of MBP was also measured by western blot analysis with antibody against human MBP and homogenates from different parts of rat brain. The results showed that each MBP isoform was expressed the most abundant in spinal cord relative to other brain tissues. To date, we also have confirmed relative expression levels of three other genes more abundantly expressed in hippocampus when compared to cerebellum with Human RNA Master. These data suggest that using Gene Filters as an initial screening tool is a good approach to filter out potential candidate genes for further validation.

Program Nr: 2117 from the 1999 ASHG Annual Meeting

Alternative splicing and polyadenylation processes occur in the gene HFE. *J-Y. Le Gall, A. Thenie, I. Gicquel, M. Ohrant, V. David, J. Mosser.* Faculty of Medicine, UPR 41 CNRS, Rennes, France.

The gene HFE responsible for hemochromatosis, which is located 4.5 Mb to HLA-A, has been cloned in 1996; it belongs to the MHC class I gene family. Several direct and indirect studies have demonstrated its involvement in both the metabolism of iron and the pathogeny of hemochromatosis. The HFE gene covers 12 kb and its structural organization, deduced from a 2.7 kb cDNA, includes 7 exons. Even if HFE might take a prominent place in the maintenance of iron homeostasis, poorly is known concerning the regulation of its expression. Hybridization experiments carried out on human multiple-tissue Northern blots revealed at least five differentially expressed mRNA. As previously described, the major transcript, 4 kb in size, is almost ubiquitously expressed, but with different expression levels among the tissues tested. We never observed a 2.7 kb mRNA, indicating that the cDNA, published at present, does not correspond to a full length molecule. Moreover the multi transcript pattern observed on Northern blots strongly suggests that HFE undergoes alternative splicing processes. Using the cDNA selection approach performed on mRNA from HeLa cells, we could clone and sequence two differentially spliced cDNA: one resulting from a proximal terminaison occurring in exon 6 and the other resulting from the splicing of several central exons. In parallel, RT-PCR experiments were carried out on the same mRNA; further hybridizations of these RT-PCR products with the different HFE exons evidenced at least ten other transcripts resulting from the splicing of one or several exons. These molecules are now under studies; the analysis of these alternative HFE mRNA might extend our knowledge of the molecular mechanisms of iron metabolism and the implication of HFE in hemochromatosis.

Program Nr: 2118 from the 1999 ASHG Annual Meeting

Characterization of human CYP17 (P450c17) promoter elements required for basal transcription in human adrenal cells. *C.J. Lin, W.L. Miller.* Pediatrics, UCSF, San Francisco, CA.

Cytochrome P450c17 is a single protein that catalyzes steroid 17a-hydroxylase and 17,20 lyase activities which are required for glucocorticoid and sex steroids synthesis, respectively. P450c17 is expressed in testicular Leydig cells, ovarian theca cells, and in the adrenal zonae fasciculata and reticularis, but not in ovarian granulosa or adrenal glomerulosa cells. Thus, characterization of factors involved in its tissue-specific transcriptional regulation may allow a better understanding of the diversity of steroid production among steroidogenic tissues. We constructed and expressed 13 promoter/reporter constructs containing from 63 to 3700 bp of CYP17 5' flanking DNA in human adrenal NCI-H295A cells, localizing essential basal elements between -184 and -227 bp. DNase I footprinting of -1 to -330 bp detected footprints at -24/-17, -70/-33, -107/-84, -178/-152 and -220/-185 bp. The -24/-17 region contains the TATA box (TTTAAAA) of P450c17 and presumably binds RNA polymerase. The -33/-70 fragment contains a putative recognition site for SF-1 and a potential site for GATA-1. Oligonucleotides containing -107/-84, -178/-152 and -220/-185 bound specifically to NCI-H295A nuclear proteins. Methylation interference assay revealed that the nuclear proteins interacts with both -107/-84 and -178/-152 through the NF-1 recognition sequence ((C/T)TGGC$(N)_6$CC$(N)_3$). Mutation of only 2 relevant bases (GG or CC) in these sequences decreased the DNA-protein binding in both fragments significantly. In the -220/185 fragment, bases -197/-195 were essential for the DNA-protein interaction but there is no factor known to bind to this sequence. Thus a NF-1-like nuclear factor binds to two of three sites in the basal enhancer sequence but the identity of the third, potentially specific factor is not yet known. Identification of this novel transcription factor binding site will permit the cloning and identification of the cognate factor.

Program Nr: 2119 from the 1999 ASHG Annual Meeting

Expression and evolution of the RBMX gene family. *P.A. Lingenfelter[1], M.L. Delbridge[2], S.S Thomas[1], J.M. Graves[2], C.M. Disteche[1]*. 1) Dept. of Pathology, Univ. of Washington, Seattle, WA; 2) Dept. of Biochemistry and Genetics, La Trobe Univ., Melbourne, Australia.

RBMX and RBMY are members of a pair of RNA-binding-motif genes with homologues on the human X and Y chromosomes (Delbridge et al., Nature Genetics, in press). RBMY, originally isolated from the Y, is specifically expressed in testis and has been implicated in male fertility. RBMX, located at Xq26, was previously shown to have a similar intron-exon structure as RBMY and to be subject to X inactivation. However, unlike RBMY, RBMX is ubiquitously expressed. FISH analysis using a probe for RBMX revealed several highly homologous sequences in the human genome. RBMX-like sequences were found at loci on human chromosomes 1, 4, 6, 9 (two loci), 11 and at a second locus on the X, which was confirmed by analysis of somatic cell hybrids retaining single human chromosomes. Sequence analysis of the RBMX-like genes revealed absence of introns, consistent with these sequences representing processed retroposons. Stop codons and deletions were found in some of these RBMX-like genes, suggesting that they are not functional. However, three of the RBMX-like genes are expressed in human tissues with different patterns between the genes, suggesting that they have evolved separate functions. These processed RBMX-like genes are conserved in primates where copies were found by PCR and FISH analyses on the corresponding chromosomes. Thus the dispersion of this family of expressed retroposons has occurred prior to the divergence of primates. The high conservation and expression of these RBMX-like genes suggest that they play important roles.

Program Nr: 2120 from the 1999 ASHG Annual Meeting

An XIST Promoter Mutation Associated with Skewed X Inactivation Alters the Pattern of Transcription Factor Binding. *M.D. Litt[1], M.A Goldman[2], R.M. Plenge[3], B.D. Hendrich[3], H.F. Willard[3], T.P. Yang[1]*. 1) University of Florida College of Medicine, Gainesville, FL; 2) San Francisco State University, San Francisco, CA; 3) Case Western Reserve University, Cleveland, OH.

Female mammals inactivate genes on one of two X chromosomes to compensate for the dosage imbalance of X-linked genes in males and females. The XIST gene, a gene transcribed only from the inactive X chromosome, has been shown to play a crucial role in the process of X chromosome inactivation. To identify cis- and trans-acting elements that regulate transcription of the human XIST gene, we analyzed the minimal promoter of the active and inactive genes by ligation-mediated PCR in vivo footprinting. Analysis of the normal XIST promoter on the inactive X chromosome revealed in vivo footprints over two potential Sp1 binding sites and a sequence of seven consecutive cytosines. No in vivo footprints were detected in the XIST promoter on the active X chromosome. These results are consistent with previous in vitro promoter analysis of the XIST gene which indicated that the Sp1 binding sites and the cytosine string are important for XIST expression. Recently, a C to G promoter mutation within the seven consecutive cytosines was identified in two unrelated families demonstrating preferential inactivation of the X chromosome harboring the mutation. In vivo footprint analysis of the mutant promoter on the inactive X chromosome revealed loss of multiple guanine contacts on the lower strand and the gain of two novel guanine contacts on the upper strand in the vicinity of the mutation. These data provide direct in vivo evidence that the functional consequences of the mutation (i.e., skewed X inactivation) may be mediated by altered transcription factor binding to the mutant XIST promoter. Therefore, we postulate that: 1) cis- and trans-acting regulatory elements in the promoter region play a key role in XIST allele selection during the process of X chromosome inactivation, and 2) the propensity to establish expression of the mutant XIST allele, and thereby inactivate the corresponding X chromosome, may be caused by a disruption or alteration of normal transcription factor binding to the mutated promoter.

Program Nr: 2121 from the 1999 ASHG Annual Meeting

Cloning of two members of the human *erg* potassium channel gene family. *N. Masuda[1, 2], S.-Y. Jeong[1, 2], H. Hashida[1, 2], T. Suzuki[1], Y. Ichikawa[2], N. Hazeki[1, 2], J. Goto[1, 2], I. Kanazawa[1, 2].* 1) CREST, Japan Science and Technology Corporation, Tokyo, Japan; 2) Department of Neurology, Graduate School of Medicine, University of Tokyo.

Potassium channels are ubiquitous membrane proteins that form the largest family of ion channels in terms of both function and structure. More than 40 genes encoding potassium channel subunits are now identified in mammals. The *erg* potassium gene families share the six-membrane-spanning architecture of the Kv class of voltage-gated potassium channels. The *erg* gene recently has become the center of considerable interest because mutations in this gene have been shown to underlie one form of a human genetic disease known as long QT syndrome, which gives rise to arrhythmias and an increased incidence of sudden death. We report here the clonings of two members of the human *erg* potassium channel gene family, *erg2* and *erg3,* which was isolated from human brain cDNA library. Human *erg2* was cloned by PCR using the primers that were designed based on the sequence of EST. Human *erg3* was isolated by PCR using the degenerate oligonucleotides directed against conserved region of the human *erg1*, rat *erg2*, and rat *erg3*. The complete coding region of *erg2* shares 90% protein homology with rat *erg2* . The human *erg3* shares 89% protein homology with rat *erg3*. The expression of human *erg2* and *erg3* in human tissues were examined by Nothern blot analysis. The human *erg2* gene and *erg3* gene were expressed exclusively in the nervous system and the sizes of the messages were approximately 3.2kb and 3.7kb respectively.

Program Nr: 2122 from the 1999 ASHG Annual Meeting

Tuberous sclerosis type 2: RNA based detection of complex splicing aberrations in the *TSC2* gene by the protein truncation test (PTT). *K. Mayer, S. Schirdewahn, W. Ballhausen, HD. Rott.* Institute of Human Genetics, Erlangen, Germany.

Mutation analyses in tuberous sclerosis (TSC) have reported a wide variety of disease causing aberrations in the two known predisposing genes, *TSC1* and *TSC2* on chromosomes 9q34 and 16p13, comprising mainly small truncating mutations distributed over the entire genes. We performed an RNA based screening of the entire coding regions of both TSC genes applying the protein truncation test (PTT) and identified a high proportion of unusual splicing abnormalities affecting the *TSC2* gene. Aberrant splicing products were initially detected as shortened polypeptides after in vitro transcription/translation, were identified through cloning and sequencing, and were confirmed by direct amplification of RT-PCR products. In two representative examples the causative changes on the genomic level turned out to be different splice acceptor mutations in intron 9 (IVS9-15G>A and IVS9-3C>G) both leading to identical exon 10 skipping and simultaneous usage of a cryptic splice acceptor in exon 10. Another splice acceptor mutation (IVS38-18A>G) destroyed a branch point sequence in intron 38 and resulted in simultaneous intron 39 retention and usage of a downstream cryptic splice acceptor in exon 39. All splice acceptor mutations described lead to different types of abnormal truncated transcripts deleting or affecting one of the known interaction domains of tuberin either for hamartin or for rabaptin5. One further patient exhibited a C>T transition in intron 8 (IVS8+281C>T) activating a consensus splice site and resulting in inclusion of a newly recognized exon in the mRNA followed by a premature stop. It is important to note that three of the reported splicing abnormalities are due to sequence changes remote from exon/intron boundaries which are described for the first time in TSC. These findings strengthen the importance of investigating intronic changes and their consequences on the mRNA level as disease causing mutations in TSC.

Program Nr: 2123 from the 1999 ASHG Annual Meeting

Recombinant Hepatitis B Surface Antigen Particles and Anionic Phospholipid Bind to Identical Sites on Apolipoprotein H (b**2-Glycoprotein I) as Revealed by Site-Directed Mutagenesis.** *H. Mehdi, A. Naqvi, M.I. Kamboh.* Human Genetics, Univ Pittsburgh, Pittsburgh, PA.

Apolipoprotein H (apoH) is an essential cofactor for the production of antiphospholipid antibodies (APA) and it has also been implicated as a possible soluble receptor for hepatitis B virus (HBV). Two naturally occurring missense mutations, Cys306Gly and Trp316Ser, in the fifth domain of apoH have been shown to disrupt the binding of apoH to anionic phospholipid (PL). We have previously shown that the binding of recombinant hepatitis B surface antigen (rHBsAg) particles with apoH is dependent on the integrity of disulfide bonds and lysine residues of apoH. Since the Gly306 mutation disrupts a disulfide bond which clusters several lysine residues and the Ser316 mutation affects the integrity of an evolutionary conserved hydrophobic sequence at positions 313-316, we hypothesized that anionic PL and rHBsAg particles would have similar binding sites on apoH and therefore these mutations would also disrupt the apoH-rHBsAg binding. We created the two mutations in apoH cDNA by site-directed mutagenesis and expressed them in COS-1 cells. The binding of recombinant apoH with rHBsAg particles and anionic PL was examined by ELISA. As compared to normal binding of the wild type apoH to rHBsAg particles and anionic PL, the mutant types apoH did not bind to either ligand. To further examine if the hydrophobic sequence at positions 313-316 is critical for binding with rHBsAg particles and anionic PL, we mutagenized the remaining three hydrophobic residues to neutral residues (Leu313Gly, Ala314Ser and Phe315Ser) and expressed them in COS-1 cells. While the Ala314Ser mutant type showed normal binding with rHBsAg particles and anionic PL, the Leu313Gly and Phe315Ser mutants showed only 38% and 9% binding with rHBsAg particles, respectivly and 27% and 17% binding with anionic PL, respectively. These data demonstrate that rHBsAg particles and anionic PL share identical binding sites on apoH. The characterization of binding sites for anionic PL and rHBsAg particles on apoH may help to understand the role of apoH in APA production and mediating the entry of HBV to hepatic cells, respectively.

Program Nr: 2124 from the 1999 ASHG Annual Meeting

Structural and functional analysis of a novel mammalian *trans*-effector of nonsense-mediated RNA decay. *J.T. Mendell[1, 2], S.M. Medghalchi[1, 2], R.G. Lake[1], H.C. Dietz[1]*. 1) HHMI and The Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; 2) These authors contributed equally to this work.

The recognition and accelerated decay of transcripts harboring premature termination codons (PTCs) is a ubiquitous feature of eukaryotic cells. This phenomenon, termed nonsense-mediated RNA decay (NMD), has been postulated to serve a protective role by preventing the translation of potentially deleterious truncated peptides. NMD has also been implicated in the regulation of steady-state levels of selected physiologic transcripts. In *S. cerevisiae*, 3 *trans*-acting factors (Upf1p-3p) are required for NMD. Strong structural and functional homologues of Upf1p have been identified in numerous species, including *S. pombe*, *C. elegans* (smg2), mouse (rent1), and human (rent1), showing that the NMD machinery, at least in part, is conserved through evolution. No counterparts for Upf2p or Upf3p have been described in other species. Here we report the initial characterization of a human homologue (RENT2) of UPF2. When compared to *S. cerevisiae* Upf2p, rent2 exhibits 21% identity and 39% similarity. Northern blot analysis demonstrated that RENT2 encodes a 5.5 kb ubiquitously expressed transcript. As an initial attempt to confirm that weak Upf2p homologues function in the NMD pathway, we identified a protein in *S. pombe* (pUpf2p) that shows the same degree of structural divergence from Upf2p as rent2. Stabilization of Ade6 nonsense transcripts in *S. pombe* following targeted disruption of pUPF2 established that the gene product is essential for NMD. These data provide compelling evidence that rent2 is the mammalian orthologue of Upf2p. Ongoing functional analyses include complementation studies in yeast and dominant negative expression studies in mammalian cells. Despite the fact that NMD is not essential in yeast or *C. elegans*, these data document strong selective pressure for conservation of Upf2p function. The study of evolutionarily conserved domains of Upf2p/rent2 may provide new insight regarding the basic mechanism and physiologic role of NMD in eukaryotic cells.

Program Nr: 2125 from the 1999 ASHG Annual Meeting

Isolation and Characterization of a new WD-repeat protein, GNB5, on Human Chromosome 21q22.3. *J. Michaud[1], M.D. Lalioti[1], J. Kudoh[2], S. Minoshima[2], N. Shimizu[2], C. Rossier[1], S.E. Antonarakis[1], H.S. Scott[1]*. 1) Div. of Medical Genetics, Geneva University Medical School and University Hospitals, Switzerland; 2) Dpt of Molecular Biology, Keio, University School of Medicine, Japan.

In order to identify candidates genes for Down syndrome phenotypes or for monogenic disorders that map to HC21, we are using exons trapped from a partial 21q22.3 bacterial contig, with homology to proteins from other species or containing specific protein motifs to pursue full-length transcripts.

We isolated a full-length cDNA mapping between D21S212 and D21S171, encoding a putative peptide of 412 amino acids with 4 guanine nucleotide-binding WD-repeats, GNB5. Northern analysis revealed two RNA species of 1.5 Kb and 2.1 Kb, the 1.5 Kb transcript being produced due to a splicing event after the stop codon. The 2.1 Kb transcript was mainly observed in fetal tissues while the shorter transcript was highly expressed in fetal liver. Both transcripts encode the same protein. Homologies, secondary structure and motif analyses of the putative GNB5 protein show the presence of 4 WD-repeats, likely to forming a propeller structure similar to known WD-repeats containing proteins. Most of these contain between 4 to 8 repeats and have diverse functions such as, among others, signal transduction and regulation of cytoskeletal assembly and cell cycle. Two alternative splicing events have been observed by RT-PCR, mainly in fetal tissues. An alternative splicing of 270 bp resulted from the omission of exons 4 and 5 which encode for two WD-repeats, and maintained the open reading frame. The second alternative splicing of 52 nucleotides removed a part of exon 3, destroying the open reading frame. From its mapping position, GNB5 is a candidate for genetic disorders linked to 21q22.3, such as one form of bipolar affective disorder, and of non-syndromic autosomal recessive deafness (DFNB10). The genomic sequence and structure of the gene have been determined to enable mutation analysis. The GNB5 gene contains eleven coding exons and a twelfth exon in the 3'UTR of the 1.5 Kb mRNA, spanning 36 Kb in total. In addition we are pursuing identification of mouse homologues to aid in functional analyses.

Program Nr: 2126 from the 1999 ASHG Annual Meeting

The regulatory function of CFTR is evolutionarily conserved. *J.E. Mickle, M.I. Milewski, G.R. Cutting.* Institute of Genetic Medicine, Johns Hopkins Univ Sch Med, Baltimore, MD.

Cystic fibrosis (CF) is a disorder of epithelial cell ion transport caused by mutations in the CF transmembrane conductance regulator (CFTR). CFTR functions as a chloride channel and a regulator of separate channels in the same cell. The importance of the regulator function in the development of the CF phenotype is unknown. Evolutionary conservation of the regulator function would indicate that it plays an important role in CFTR-mediated ion transport. The most distant cousin of human CFTR cloned to date is from the killifish (*Fundulus heteroclitus*). Killifish (kf) and human (h) CFTR share 60% amino acid identity. In this study, we expressed kfCFTR in human cells to determine whether it regulates separate chloride channels. To assess whether kfCFTR was processed in the same fashion as human CFTR, we transiently expressed kfCFTR in human embryonic kidney 293 cells. Cell lysates were immunoprecipitated with a monoclonal antibody specific for the C-terminus of kfCFTR followed by protein kinase A radiolabeling with ^{32}P, SDS-PAGE and autoradiography. Similar to mature hCFTR, kfCFTR was detected as a single band with an approximate molecular mass of 190 kDa, indicating the kfCFTR is fully processed in human cells. The function of kfCFTR was studied in IB3-1 human CF airway epithelial cells by whole cell patch clamp. To facilitate detection of transfected cells, kfCFTR was co-expressed with GFP at a 10:1 ratio (kfCFTR:GFP). Cells were bathed in CPT-cAMP(200mM) and forskolin(5mM), conditions that maximally activate hCFTR. Whole cell recordings reveal that expression of kfCFTR in IB3-1 cells is associated with two chloride currents: a non-rectifying glybenclamide-sensitive component characteristic of the intrinsic channel of CFTR, and an outwardly-rectifying DIDS-sensitive component indicative of CFTR-mediated regulation of separate chloride channels. These data suggest that CFTR-mediated regulation of other channels is evolutionarily conserved and therefore likely to be essential for normal epithelial ion transport.

Program Nr: 2127 from the 1999 ASHG Annual Meeting

The human EZH2 gene: Genomic organization and revised mapping in 7q35 within the critical region for malignant myeloid disorders. *C. MIGNON[1], C. CARDOSO[1], G. HETET[2], B. GRANCHAMP[2], M. FONTES[1], L. COLLEAUX[1]*. 1) INSERM U491, Faculte de Medecine de la Timone, Marseille, FRANCE; 2) INSERM U409 and Centre de Recherche Claude Bernard, Faculte de Medecine Xavier Bichat, Paris, FRANCE.

The EZH2 gene is a human homolog of the Drosophila Polycomb-group (Pc-G) gene Enhancer of zeste, a crucial regulator of homeotic gene expression. Several lines of evidence suggest a critical role for the EZH2 protein during normal and perturbed development of the hematopoietic and central nervous systems. Indeed, the EZH2 protein has been shown to associate with the Vav proto-oncoprotein and with the XNP protein, the product of a mental retardation gene. The EZH2 gene was previously reported to be located on chromosome 21q22 and was proposed as a candidate gene for some characteristics of the Down syndrome phenotype. We report here the genomic structure and fine mapping of the EZH2 gene. We demonstrate that the functional gene actually maps to chromosome 7q35 and that the sequence previously isolated from a chromosome 21 cosmid corresponds to a pseudogene. Finally, the nature of the EZH2 protein and its mapping to the critical region for malignant myeloid disorders lead us to propose the EZH2 gene to be involved in the pathogenesis of 7q35-q36 aberrations in myeloid leukemia.

Program Nr: 2128 from the 1999 ASHG Annual Meeting

Charaterization of the mouse Ccs gene and mapping the human counterpart. *S.D.P. Moore, M.M. Chen, D.W. Cox.* Medical Genetics, University of Alberta, Edmonton, Alberta, Canada.

Copper does not exist in a free state within cells, but is always bound to metalloproteins. Specific metallochaperones escort copper to numerous targets within the cell, providing protection from the toxic effects of intracellular free copper. The known components of the intracellular copper transport pathway are identical in yeast and mammals. Yeast Lys7p (human CCS (copper chaperone for superoxide dismutase)) delivers copper to superoxide dismutase (SOD1), yeast Cox17p (human COX17) delivers copper to cytochrome c oxidase in the mitochondria via Sco1p (human SCO1), and yeast Atx1p (human ATOX1) delivers copper to the P-Type ATPase, Ccc2p (human ATP7A and ATP7B). Many, but not all, of these genes have been characterized in yeast, mouse, and human. To further characterize murine Ccs we have cloned it by screening a murine cDNA library with a human CCS clone, providing both the coding region and the 5' and 3' UTRs. We obtained a 1174 base pair (bp) cDNA with an 825 bp open reading frame. The open reading frame putatively translates a 274 amino acid protein that is 86.9% identical to human CCS. A comparison of the hydrophobicity plots confirms both the conservation between hydrophobicity and the cytosolic nature of the conserved proteins. In addition, we have mapped human CCS to 11q13, utilizing a hamster/human radiation hybrid panel. CCS has been demonstrated to be an integral part of the disorder amylotrophic lateral scerosis (ALS). Further characterization of mouse and human CCS will aid in studying this disorder.

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Program Nr: 2129 from the 1999 ASHG Annual Meeting

Isolation and characterization of a novel human transcript in the Familial Dysautonomia candidate region on human chromosome 9q31. *J. Mull[1], M. Leyne[1], S.P. Gill[1], C.B. Liebert[1], C.M. Robbins[2], H.W. Pinkett[2], I. Makalowska[2], C. Maayan[3], A. Blumenfeld[3], F.B. Axelrod[4], M. Brownstein[2], B.P. Chadwick[1], S.A. Slaugenhaupt[1]*. 1) Molecular Neurogenetics, Harvard Medical School, Boston, MA; 2) National Institutes of Health, Bethesda, MD; 3) Hadassah University Hospital, Jerusalem, Israel; 4) New York University Medical School,New York, NY.

Familial Dysautonomia (FD; Riley Day syndrome or hereditary sensory neuropathy type III) is an autosomal recessive disorder characterized by pervasive sensory and variable autonomic dysfunction. FD affects the development of sensory, sympathetic and some parasympathetic neurons. The gene defect is found almost exclusively in the Ashkenazi Jewish population where it has a carrier rate of 1 in 30 and an incidence of 1 in 3600 live births. We used a combination of linkage analysis, haplotype analysis and physical mapping to narrow the candidate region of the FD gene to a 0.5 cM region on human chromosome 9q31. Exon trapping, cDNA selection and genomic sequencing of overlapping BACs, YACs, and cosmids were used to isolate a novel transcript, CG-8, from the FD candidate region. Northern analysis revealed a ubiquitous 2kb transcript with highest expression in adult testis. An open reading frame was identified that encodes a 181 amino acid protein with 30% amino acid identity to the glycine cleavage system H protein of Yeast and bacteria. A total of six exons were identified for CG-8 by direct genomic sequencing, covering approximately 6.5kb. The full coding region of CG-8 was sequenced from genomic DNA and reverse transcribed RNA in four affected, and two non carrier individuals. A 4 bp deletion was identified in the 3' UTR of CG-8. Further analysis showed the 4bp deletion in two non-carrier individuals indicating that the sequence change was not responsible for the pathogenesis in FD. Although no coding mutaions were found, a promoter or non-coding mutation remains a possibility and is currently under investigation.

Program Nr: 2130 from the 1999 ASHG Annual Meeting

Analysis of *GFR*a*1* polymorphism and splice variants; effect on GFRa1/GDNF/RET complexes. *S. Myers[1], D. Worley[2], G. Cote[3], L. Mulligan[1].* 1) Dept Pathology, Queen's University, Kingston, ON, K7L 3N6, Canada; 2) BIOGEN, Inc., Cambridge, MA 02142; 3) M.D. Anderson Cancer Center, Houston, TX 77030.

The signaling complex of the RET receptor tyrosine kinase, GFRa1 and GDNF, is recognized as essential for normal sympathetic enervation of the gut. Mutations throughout the *RET* gene have been found to be responsible for Hirschsprung disease (HSCR) and mutations in *GDNF* are also believed to contribute to this disease. No mutations have been identified in the *GFRa1* gene in HSCR patients but five polymorphisms are present in this gene which result in amino acid changes. The variable penetrance and expressivity of HSCR suggest that one or more loci may modify the HSCR phenotype associated with *RET* mutations. One possible modifier could be functional differences associated with these different polymorphic isoforms of GFRa1. In order to test this we have generated *GFRa1* cDNA clones containing each polymorphic allele at two of these sites, 253T/A (Y85N) and 1081A/G (T361A), using *in vitro* mutagenesis. We have transfected these constructs into E293 cells and assayed for the amount of GFRa1 protein produced and translocated to the cell surface by western analysis of PIPLC cleaved GFRa1, and FACS analysis using anti-GFRa1 antibodies. Our data show that GFRa1 proteins containing either of the two amino acids at codons 85 or 361 are expressed and translocated to the cell surface with similar efficiency. FACS analysis of GDNF binding to E293 cells expressing these different forms of GFRa1 further suggests that binding efficiency is similar among the three forms. Binding of RET to GFRa1/GDNF complexes also appears to be equivalent for the GFRa1 forms using a similar FACS analysis. In addition to the polymorphisms, a splice variant of GFRa1, resulting in the addition of five amino acids, has been identified. We have generated a GFRa1 construct containing these extra amino acids. In the context of the GFRa1 polymorphisms described, we are currently comparing the expression and binding of these GFRa1 proteins in E293 as outlined above.

Program Nr: 2131 from the 1999 ASHG Annual Meeting

Splice variants, expression and identification of the murine orthologue of the retina-specific L-type calcium channel *CACNA1F* gene, which is mutated in patients with incomplete CSNB. *M.J. Naylor[1], D.E. Rancourt[2], N.T. Bech-Hansen[1]*. 1) Medical Genetics, University of Calgary, Calgary, AB, Canada; 2) Oncology, University of Calgary.

Patients with incomplete X-linked CSNB (iCSNB) have mutations in *CACNA1F*, an L-type calcium channel alpha-1-subunit gene mapping to Xp11.23 (Nature Genet. 19:260-263 and 264-267, 1998). More than 18 different mutations of this gene have been identified by our laboratory in patients with iCSNB and in several patients reported to have Aaland Island eye disease (AIED) or AIED-like disease, suggesting that iCSNB and AIED/AIED-like conditions are allelic forms of the *CACNA1F* gene. In studying the genomic organization of *CACNA1F*, several splice variants were observed across the cDNA of this gene. For example, variants involving two forms of exon 1 and five forms of exon 2 were identified; several combinations of these exons are predicted to yield complete splice-variant proteins. Mutations in three families with incomplete CSNB were located in the unique portion of the larger variant of exon 2, suggesting a significant role for this particular exonic variant. Our findings suggest a prototypical cDNA sequence of *CACNA1F* which produces a protein of 1977 amino acids. In further studies, we have identified the sequence of the full-length murine homologue of *CACNA1F*, *Cacna1f*. Mapping of this homologue to a mouse BAC which also contained the mouse synaptophysin gene established that the identified *Cacna1f* gene is the orthologue of the gene for incomplete CSNB. Furthermore, *in situ* hybridization using mouse probes of *Cacna1f* on mouse retinal sections showed expression of *Cacna1f* in the outer and inner nuclear layers, and the ganglion cell layer of the retina. Having identified the full-length mouse orthologue of *CACNA1F* and the expression pattern of *Cacna1f* in the retina, we are in a position to construct an animal model of iCSNB. This research was supported in part by the RP Research Foundation (Canada) (NTBH, DER), the Odd Fellow Rebekah Visual Research Student Awards (MJN).

Program Nr: 2132 from the 1999 ASHG Annual Meeting

Cloning and sequence analysis of Drosophila, Xenopus and Chicken orthologs of the human Ubiquitin-Fusion-Degradation 1 (UFD1L) gene. *G. Novelli[1], A. Ratti[2], M. Bozzali[2,3], A. Botta[1], F. Sangiuolo[1], A. Pizzuti[2,3], B. Dallapiccola[1,3].* 1) Dpt Biopathology Tor Vergata University and CSS Mendel Institute, Rome,Italy; 2) Institute of Neurology, University of Milan, Milan, Italy; 3) IRCCS CSS San Giovanni Rotondo, Italy.

UFD1L gene encodes the human homolog of the yeast ubiquitin fusion-degradation 1 protein (UFD1p), which in yeast is active in a ubiquitin-dependent proteolytic pathway. Depletion of this protein also affects polyadenylation activity, resulting in defective RNA processing. The UFD1L mRNA is expressed in brain, lungs, branchial arches, cardiac outflow tract, lens, otocyst and limb bud. UFD1L is deleted in DiGeorge syndrome (DGS) and velocardiofacial syndrome (VCFS) patients and, based on expression profile, is considered at present a strong candidate for this disease. In order to elucidate the biochemical role of the gene product during invertebrate and vertebrate development, we characterized the UFD1L orthologs from D.melanogaster, X.laevis and Chick. The nucleotide homology of the Drosophila and Xenopus orthologs compared to the mammalian cDNAs is low. However when the sequence got translated into protein the overall homology raised to more than 84% (60% identity) of the mammalian cDNA. The maximum homologies rest in the central and COOH portions of the protein, while at NH2 end the homology is low. The chicken protein has a 95% identity to the mammalian proteins. The gene structure is conserved in human, mouse and Drosophila. This study demonstrates that the biological function of the UFD1L gene product is evolutionary conserved and provides new insight into understanding the DGS pathogenesis. Work supported by an Italian Telethon grant (E.723) and (364/bi).

Program Nr: 2133 from the 1999 ASHG Annual Meeting

Genomic Organization and Immunofluorescence Studies of Human Sarcospan. *K.F. O'Brien[1], L.M. Kunkel[1,2]*. 1) Department of Genetics, Harvard Medical School, Boston, MA; 2) HHMI/Division of Genetics, Children's Hospital, Boston, MA.

Sarcospan has recently been identified as the 25 kDa component of the dystrophin associated protein complex (DAPC). Within the DAPC, sarcospan has been shown to be most closely associated with the sarcoglycan complex (a-, b-, g-, and d-Sgn). Each of these four sarcoglycans is known to cause limb girdle muscular dystrophy (LGMD2-D, E, C and F, respectively) when mutated. While the sarcoglycans share several structural features, sarcospan is homologous to proteins from the tetraspanin family (TM4SF).

When muscle biopsies taken from Duchenne Muscular Dystrophy (DMD) patients are analyzed by immunofluorescence for the presence of the sarcoglycan complex, staining is often found to be reduced. Similarly, primary mutations in any one of the sarcoglycan genes lead to secondary loss of staining for the other members of the sarcoglycan complex. To elucidate sarcospan's function in the muscle membrane we chose to examine sarcospan's presence in muscle biopsies from a variety of muscular dystrophy patients. To do this, we have developed a human sarcospan specific antibody with which we have analyzed a number of LGMD, DMD and congenital muscular dystrophy (CMD) patient samples. Sarcospan staining is decreased in cases where there is loss of the sarcoglycan complex and/or dystrophin.

There are still patients for whom mutations have not been identified in any of the genes known to cause muscular dystrophy. Since sarcospan is intimately associated with the sarcoglycan complex, it is conceivable that mutation of the sarcospan gene would lead to muscular dystrophy. We have determined the complete genomic organization of the human sarcospan gene, and have designed a primer set to specifically amplify sarcospan exons from human genomic DNA. These are currently being used to screen DNA samples from patients with muscular dystrophies that are not caused by mutations in any of the known genes, as well as patients with diseases linked to chromosome 12p11, sarcospan's genomic location.

Program Nr: 2134 from the 1999 ASHG Annual Meeting

Characterization of the *GFRA3* locus and investigation of the gene in Hirschsprung disease. *C.I. Onochie, L.M. Korngut, D. Michaud, S.M. Myers, L.M. Mulligan.* Department of Pathology, Queen's University, Kingston, ON, K7L 3N6, Canada.

The GFR alpha family of GPI-linked cell membrane proteins act as adapter molecules in binding of the GDNF family of soluble neurotropic factors to the cell surface RET receptor. This system is essential for development of many neural crest derived cell types and the kidney. Mutations in RET and in two of its ligands, GDNF and NTN, play a role in the development of Hirschsprung disease (HSCR), a congenital absence of the enteric ganglia. Mutations have not been found in the *GFRA1* gene in HSCR samples, however, other members of the GFR alpha family remain candidates. One such gene is *GFRA3*, which is highly expressed in the peripheral nervous system, developing nerves, heart, lung, and kidney. We have characterized the *GFRA3* locus and investigated the occurrence of sequence variants of this gene in a control population and in a panel of HSCR patients. We have shown that *GFRA3* spans 8 coding exons on chromosome 5q31. The gene structure and organization is conserved with those of GFRA1 and 2. Using a combination of single strand conformation polymorphism (SSCP) analysis and direct sequencing, we have identified polymorphic variants in exons 4, 5, 7 and 8 of *GFRA3* in both normal controls and HSCR patients. We are currently investigating 3 additional sequence variants in *GFRA3* exons 1, 5 and 7. The expression pattern of GFRA3, its predicted role in differentiation of embryonic cell types, and its involvement in RET mediated signaling, all suggest that GFRA3 could be a candidate for mutations in HSCR. The current study will elucidate the potential role of GFRA3 in this disease.

Program Nr: 2135 from the 1999 ASHG Annual Meeting

Cloning, expression analysis, genomic localization, and functional characterization of a novel Class I cytokine receptor expressed in cells of lymphoid origin. *J. Parrish, S. Presnell, D. Conklin, A. Hammond, C. Sprecher, S. Schrader, W. Xu, K. Madden, C. Brandt, J. Gross, J. Johnston, S. Mudri, A. Nelson, S. Dillon, K. Hambly, H.-P. Ren, F. Raymond, T. Whitmore, M. Maurer, D. Foster.* Stem Cell Biology, ZymoGenetics, Inc., Seattle, WA.

We have identified a novel Class I cytokine receptor, zalpha11, which is expressed in lymphoid tissues. This receptor contains the hallmark motifs of its class, including two pairs of conserved cysteine residues and a conserved "WSxWS" motif in its extracellular domain, and conserved Box I and Box II signaling motifs in its intracellular domain. Northern analysis indicates low-level expression in spleen, thymus, lymph node, bone marrow, and peripheral blood leukocytes; expression appears to be upregulated in a Burkitt lymphoma cell line (Raji). RT/PCR and flow cytometry suggest expression of zalpha11 on resting and activated B cells and on activated T cells. Immunohistochemistry using a polyclonal antibody raised against soluble zalpha11 localizes the receptor to a subset of cells in spleen and thymus, and shows upregulation in PMA-activated HL-60 cells. Using RH panel mapping, zalpha11 was localized to 16p11, a region associated with susceptibility to inflammatory bowel disease. In order to assess the signaling capability of zalpha11, a chimeric receptor was constructed which consisted of the extracellular and transmembrane domains of Mpl, which forms a homodimer in the presence of thrombopoietin (TPO), fused with the cytoplasmic domain of zalpha11. This construct was transfected into the murine IL3-dependent cell line BaF3, and proliferative capacity was measured in the presence of TPO without IL3. Cells transfected with the chimeric receptor were able to proliferate in response to TPO, indicating that homodimeric zalpha11 is capable of delivering a proliferative signal to BaF3 cells. Given the lymphoid expression pattern and signaling capability of zalpha11, its ligand can be expected to be a potent lymphopoietic factor.

Program Nr: 2136 from the 1999 ASHG Annual Meeting

DAX1, SF1 and SOX9: Evolutionary Rates of Sequence Variation May Reflect Relative Positions in the Mammalian Sex Determining Pathway. *M. Patel[1], E. Vilain[2], K. Dorman[3], Y.-H. Zhang[4], B.-L. Huang[4], A.P. Arnold[1], J. Sinsheimer[2,3], E.R.B. McCabe[4].* 1) Depts. of Physiological Science; 2) Human Genetics; 3) Biomathematics; 4) Pediatrics, UCLA, Los Angeles, CA 90095-1752.

Mammalian sex determination is dependent upon the Y chromosome gene, SRY. SRY acts to trigger male sex determination in mammals, presumably by regulating downstream genes in the sex determining cascade. DAX1 is a candidate gene for dosage sensitive sex reversal seen in XY females with tandem duplications of Xp21.3. Investigators speculate that DAX1 and SRY are antagonistic, suggesting that they may be involved at the same level of the sex determining pathway. These genes would act to regulate the activity of downstream genes such as SF1 and SOX9 in a dosage dependent manner. Sequence comparisons of SRY and DAX1 across primate species suggest both genes may be rapidly evolving. Here we compare the rates of evolution of SRY, DAX1, SF1 and SOX9 in primates. A measure of evolutionary divergence is the number of nonsynonymous substitutions. We sequenced the coding regions of SRY, DAX1, SF1 and SOX9 from rhesus genomic DNA and calculated the number of nonsynonymous (Ka) and synonymous (Ks) substitutions. Ka values for SRY, DAX1, SF1 and SOX9 were 0.051+/-0.011, 0.012+/-0.003, 0.013+/- 0.004 and 0.00+/- 0.00 respectively. Ks values were 0.102+/-0.03, 0.025+/-0.009, 0.055+/-0.012 and 0.029+/-0.008 respectively, suggesting pressure for SRY, DAX1 and SF1, but not SOX9, to acquire nonsynonymous changes. Ka/Ks ratios were 0.497 for SRY, 0.491 for DAX1, 0.227 for SF1 and 0.000 for SOX9. We conclude that DAX1 is evolving more rapidly than SOX9 and at a rate similar to SRY, perhaps to maintain a functional relationship with SRY. SF1 seems to evolve at an intermediate rate, perhaps reflecting an intermediate position in the sex determination hierarchy. Our data suggest DAX1 is involved early in the sex determinating pathway and may functionally interact with SRY either directly or indirectly.

Program Nr: 2137 from the 1999 ASHG Annual Meeting

Genomic structure of a retinal expressed candidate gene for dominant optic atrophy (Kjer type). *U.E.A. Pesch, C. Alexander, B. Wissinger.* Molecular Genetics Lab, University Eye Hospital, Tuebingen, Germany.

Dominant optic atrophy, Kjer type, is the most common form of hereditary atrophies of the optic nerve. Patients suffer from a progressive loss of visual acuity and colour vision disturbance from early childhood onwards. Pathological examination of patients eyes demonstrated a loss of retinal ganglion cells. Linkage studies showed the OPA1 gene to be located in a 1-2 cM intervall on chromosome 3q28-q29. 7 ESTs have been fine mapped so far to the disease critical region. EST SHGC37414 was found to belong to a very large THC (tentative human consensus sequences) Hs.32473 consisting of 89 different cDNA-clones and to be expressed in retinal tissue. The full length transcript was cloned by Nagase et al., 1998. BLAST analysis of the coding region revealed homology to dynamin. A GTP-binding site was predicted by various motiv search programs. Our aim was to determine the genomic structure of the KIAA0567 gene by comparing the full length cDNA with the genomic sequence. A genomic PAC clone containing the SHGC37414 sequence was subcloned and sequenced. The sequences were assembled into contigs using the staden software package and screened for exons with the KIAA0567 cDNA sequence. Primers were designed in order to experimentally verify the virtually identified 16 intron/exon boundaries. Exon hopping by expand PCR was applied to identify another 10 intron/exon junctions. The KIAA0567 gene seems to have a very complex structure of at least 25 exons. Mutation screening in affected individuals is currently in progress.

Program Nr: 2138 from the 1999 ASHG Annual Meeting

Identification of a novel *DMD* exon that is alternatively-spliced in retinal isoform (Dp260) of dystrophin. *D.M. Pillers[1,2], J. Pang[1], R.G. Weleber[2,3], S.A. Tokarz[1,2], S.M. Rash[1], N.M. Duncan[1]*. 1) Dept Pediatrics, Oregon Health Sci Univ, Portland, OR; 2) Dept Molecular and Medical Genetics, Oregon Health Sci Univ, Portland, OR; 3) Dept Ophthalmology, Oregon Health Sci Univ, Portland, OR.

Introduction: The *DMD* gene spans more than 2.6 Mbp but the coding sequence comprises only 14 kb and 79 exons. The *DMD* gene does not follow the "one gene - one protein" paradigm, as there are at least five described protein products, or isoforms, of dystrophin: Dp427, Dp260, Dp140, Dp116, and Dp71. A number of new exons have been identified as isoform-specific exons, bringing the total of known exons to 86. These exons occur in the first exon position of the isoforms, and are either isoform or tissue-specific. Other than characterizing patterns of alternative splicing at the 3' end of dystrophin, the remaining sequence of the various dystrophin isoforms has been inferred by homology with Dp427. **Purpose:** The purpose of our study was to sequence the complete Dp260 isoform to determine if all of the known exons were expressed as a guide to identifying features of the isoform that might predict unique functional roles. **Methods:** Dp260 isoform was amplified by long RT-PCR from mouse mRNA using primers specific to the unique Dp260 first exon R1 (forward) and to the junction of exons 78 and 79 (reverse). The product was sequenced in two directions and the sequence was compared to Dp427 in GenBank. **Results:** An amplimer of predicted size was obtained. Sequence analysis confirmed the presence of exon R1 followed by exons 30 through 79 of Dp427, in predicted order. An additional 36 bp exon not previously defined was also identified. The open reading frame was preserved through the new exon. **Conclusions:** The *DMD* gene is both large, and complex, and there may be many aspects of its expression yet to be elucidated. We have identified a new exon that appears to be uniquely expressed in retina. The exon is also unique in that it is in the center of the message, and not at either the 5' or 3' ends as occurs with other alternatively spliced exons of dystrophin. Further characterization of this exon may lead to important insight into potential key roles that dystrophin isoform Dp260 plays in retinal electrophysiology.

Program Nr: 2139 from the 1999 ASHG Annual Meeting

Characterization of *COX17*,a component of the mitochondrial copper transport pathway. *F. Punter, D. Adams, D.M. Glerum.* Department of Medical Genetics, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

Cytochrome c oxidase (COX), the fourth and final electron-accepting enzyme complex of the mitochondrial respiratory chain, catalyzes the transfer of electrons to molecular oxygen. Dysfunctional COX hinders cellular respiration and ATP synthesis, giving rise to a number of human disorders. One important aspect of COX biogenesis involves the incorporation of copper into the apoenzyme, where the heavy metal acts as an essential electron-accepting prosthetic group. From previous studies in yeast, the mitochondrial copper transport pathway is thought to consist of two proteins, Cox17p and Sco1p. Functioning as a copper shuttle, Cox17p is believed to transport copper from the cytosol to Sco1p, which is embedded in the inner membrane of the mitochondrion.

To elucidate the human mitochondrial copper transport pathway and its role in COX biogenesis and human COX deficiency, we are characterizing the human *COX17* gene. Southern blot analysis of human genomic DNA has shown that *COX17* is present in multiple copies in the human genome. BAC and P1 clones harbouring *COX17* are currently being sequenced in order to characterize the two putative copies of *COX17*. Using radiation hybrid mapping, we have identified one of the *COX17* loci at chromomsome 13q14-21. Hybridization of the *COX17* cDNA to a multiple tissue Nothern blot shows one ubiquitously expressed *COX17* transcript. We are in the process of determining which of the genomic loci expresses a functional *COX17* gene product. This study, which will result in the characterization of the *COX17* gene in humans, is an important first step in the delineation of the human mitochondrial copper transport pathway and and its role in human COX deficiencies.

Program Nr: 2140 from the 1999 ASHG Annual Meeting

Molecular characterization of a Rieger syndrome mutation in PITX2. *I. Saadi[1], K.P. Murphy[2], A.F. Russo[1,3]*. 1) Genetics PhD Program; 2) Department of Biochemistry; 3) 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. A lysine residue at position 50 of the PITX2 homeodomain (K50) imparts the bicoid-type binding specificity (TAATCC) to its homeodomain. This lysine residue is mutated to an arginine (K88R) in a Rieger patient. Although lysine to arginine would normally be considered a conservative change, our studies show that the K88R mutant protein is severely defective in its binding and transactivation properties. We have computer-modeled the PITX2 homeodomain-DNA complex based on the NMR structure of the antennapedia homeodomain-DNA complex and show that the defective binding is likely due to steric hindrance of the arginine side-chain. We also wanted to know if an arginine at position 50 of the homeodomain would result in a different binding specificity. Selection and amplification binding (SAAB) assay shows that the K88R mutant protein does not have a preferred DNA binding specificity. However, the K88R mutant acts in a dominant-negative fashion when co-transfected with wildtype PITX2, suggesting that it forms nonfunctional dimers with wildtype PITX2. This may explain the autosomal dominant nature of Rieger syndrome wherein a single allele variant confers the phenotype, and this work suggests an approach to understanding the genotype-phenotype correlations found for other transcription factors.

Program Nr: 2141 from the 1999 ASHG Annual Meeting

Transthyretin gene: transcription pattern in livers and heart from transplanted patients with hereditary Amyloidosis. *F. Salvi[1], E. Manzati[2,3], Y. Ando[4], A. Ferlini[2,3]*. 1) Div di Neurologia, Ospedale Bellaria, Bologna, Italy; 2) Istituto di Genetica Medica, Universita' di Ferrara, Italy; 3) Neuromuscular Unit, Imperial College School of Medicine, Hammersmith Campus, London ,UK; 4) Department of Human Genetics, Yamamoto University, Japan.

Transthyretin (TTR) gene missense mutations are associated with hereditary Amyloidosis, an autosomal dominant disease mainly characterised by peripheral neuropathy and cardiomyopathy. The only therapeutic approach for this disease is the liver transplant, eliminating the plasma aliquot of the mutated TTR. We performed transcription analysis on 7 livers and 1 heart from transplanted patients with documented TTR mutations (Met30, Pro36,Ala47,Gln89), searching for additional/alternative transcripts. Transcription studies on patients tissues and normal adult liver have been performed using a 5 RACE-PCR approach, resulted in the amplification of two transcripts: the first corresponding to the canonical transcription starting site (TTRP1, nucleotide 583 in TTR genomic sequence accession number m11844), the second one representing the RAT-like transcription starting site (TTRP2, transcription starting site at nucleotide 499). Northern blot hybridisation using a 80 bp probe containing specific for the TTRP2 transcript, confirmed the presence of the TTRP2 message. The two transcripts coexist in normal and patients livers. Surprisingly, the heart carrying the TTR89 mutation showed the two TTRP1 and TTRP2 transcripts as well as an additional longer transcript. We demonstrated the presence of two alternative TTR messages,due to the presence of two active transcription starting sites. Livers from controls and transplanted patients display an identical transcription pattern, suggesting that TTR mutations do not apparently affect the transcription pathway. Interestingly, the heart carrying the TTRGln89 mutation showed both the TTRP1 and TTRP2 transcripts and an additional longer product. This is unexpected, given the absence of the TTR transcription/expression in normal adult heart. Further investigations are needed for establishing a possible role of these transcripts in the pathogenesis of TTR-related amyloidotic cardiomyopathy.

Program Nr: 2142 from the 1999 ASHG Annual Meeting

Cloning and genomic structure of OTX2, a candidate gene for Congenital Microphthalmia. *M. Sarfarazi, I. Stoilov, E.F. Percin.* Molecular Ophthalmic Genetics Laboratory, Surgical Research Center, University of Connecticut Health Center, Farmington, CT.

OTX2 is the human cognates of Drosophila head gap gene, orthodenticle. This gene is involved in early specification of rostral neuroectoderm and sense organ development. Based on its proposed function as a regulator of early forebrain development, OTX2 is a good candidate for many developmental eye defects including microphthalmia. We have recently screened 9 multiply affected families with congenital microphthalmia for possible linkage to the 14q21-q22 region, of whom 3 showed homozygosity for an area that contains the OTX2 gene. We therefore embarked on cloning of this gene as a candidate for this condition. Using RACE, this gene was cloned from both human retinal and brain cDNA libraries. The genomic region amplified by Long Range (LR) PCR and the intron-exon junctions were determined by comparative sequence analysis of cDNA and genomic DNA. The 5' flanking region recovered by LR-PCR using primers designed from the mouse Otx2 gene. Additional 5' sequences obtained by upstream genome walking experiments. The OTX2 gene spans 4,991 bp, including 1,137 bp of 5' region, 3 exons, 2 introns and 3' UTR. All intron-exon junctions conform to the GT/AG rule. Exon II encodes the bicoid-like homeodomain and resides within a CpG island that is marked by a cluster of six HpaII restriction sites. Comparative analysis revealed that human OTX2 gene is highly conserved and resembles that of the mouse Otx2 gene. The two cis-acting elements controlling expression of the mouse Otx2 gene in mesencephalic neural crest cells are conserved and present at similar positions in the 5' flanking region of the human OTX2 gene. The obtained sequences and genomic structure of OTX2 gene were used to screen the affected members of 3 above-mentioned families and 7 other sporadic cases for possible mutations. Sequence analysis of the entire coding regions found no mutations in any of these subjects. Since families showing homozygosity for the OTX2 region have a nuclear structure, we concluded that this observation is either coincidental or, another regional gene is involved in the etiology of this phenotype.

Program Nr: 2143 from the 1999 ASHG Annual Meeting

HUMAN TYPE XVIII COLLAGEN (COL18A1) GENE: STRUCTURAL ORGANIZATION, IDENTIFICATION OF NEW SNPs, AND ITS EXCLUSION AS THE KNOBLOCH SYNDROME GENE. *A.L. Sertie[1], V. Sossi[2], M.R. Passos Bueno[1], C. Brahe[2].* 1) Dept of Biology, University of Sao Paulo, Sao Paulo, Brazil; 2) Inst of Medical Genetics, Catholic University, Rome, Italy.

The gene responsible for Knobloch syndrome (KS) - an apparently rare autosomal recessive disorder characterized by high myopia, vitreoretinal degeneration with retinal detachment, macular abnormalities and occipital encephalocele - was mapped to 21q22.3, within a candidate region of 1.6Mb. COL18A1, the gene which encodes type XVIII collagen, lies in this interval and was considered a good positional and functional candidate gene for KS. We report here the characterization of most of the genomic structure of COL18A1 gene throughout the amplification and/or sequencing of the gene introns using a set of 45 flanking exonic primer pairs deduced from the human cDNA COL18A1 sequence and taking into account the organization of the mouse Col18a1 gene. The data showed that COL18A1 consists of 43 exons spanning over 102kb of genomic DNA and is very similar to the mouse gene. Although 45% of the donor splice junctions are identical to the consensus sequence G/GT(AG)AGT, 84% of the acceptor splice sites agree with the consensus sequence (TC)nN(CT)AG/G. These results suggest that the conservation of the acceptor sequence is very critical for a correct splice in this gene. We also have identified two new SNPs in this gene which occur in a relatively high frequency in the Caucasian population: a G to A exchange at the +58 nucleotide of intron 6 and an A to G exchange at the +54 nucleotide of intron 17. These markers were analysed in Brazilian patients with KS revealing recombination between it and the disease gene. In addition, exons 4 to 7 of COL18A1 were screened for mutation and no changes were identified. Taken together, our data provide strong evidence that COL18A1 gene is not the gene responsible for KS. Furthermore, the genomic structure of COL18A1 and its polymorphic markers will certainly be important for the study of other genetic diseases mapping to distal 21q. Supported by: FAPESP, HHMI, CNPq, PRONEX.

Program Nr: 2144 from the 1999 ASHG Annual Meeting

Human lysosomal sialidase: analysis of the gene promoter. *V. Seyrantepe[1], C. Richard[1], J. Engert[2], T. Hudson[2], A.V. Pshezhetsky[1]*. 1) Dept of Medical Genetics, Ste-Justine Hospital, Montreal University; 2) Montreal General Hospital, McGill University, Montreal, Quebec, Canada.

The inherited deficiency of sialidase (neuraminidase) causes a lysosomal storage disease, sialidosis characterized by cherry-red macular spots, myclonus, skeletal dysplasia, mental retardation and hepatosplenomegaly. In addition to its well-established role in the intralysosomal catabolism of sialilated glycoconjugates, sialidase is also involved in the cellular signaling. In particular, sialidase of T lymphocytes converts group specific component (Gc) protein into a macrophage activating factor and is also required for the production of cytokine IL-4, the potent regulator of many homeopathic and nonhomeopoetically derived cells and tissues. Activation of T-lymphocytes is associated with the increase of sialidase activity on their surface due to the up-regulation of sialidase gene. In order to understand the mechanism of this activation, we analyzed the promoter region of human sialidase gene for the presence of consensus sequence for upstream regulatory elements. Transcription start sites of sialidase mRNA were identified by 5'RACE PCR experiments using total RNA from fibroblasts and placenta and confirmed by ribonuclease protection assays. To identify essential promoter elements in sialidase gene, we generated several constructs by cloning 5-end fragments of sialidase gene (nucleotides 1 to 2200) into the pBLCAT6 vector in front of a reporter, chloramphenicol acetyl transferase (CAT) gene. Transient transfection of these constructs into COS-7 cells and subsequent analysis of CAT activity demonstrated that the region of sialidase gene ~300 bp upstream the initiation codon has minimal promoter activity and the region ~550 bp upstream the initiation codon has dominant promoter activity containing all essential promoter elements. Larger reporter construct, within 2.2 kb of the upstream sequence had lower promoter activity suggesting the presence of the potential elements for down-regulation of gene expression. These findings elucidate the basic mechanism for the regulation of sialidase gene expression and may explain its induction observed in the immune cells.

Program Nr: 2145 from the 1999 ASHG Annual Meeting

identification of a novel gene and its exclusion as a candidate for the urofacial (ochoa) syndrome (ufs) gene on chromosome 10q23-10q24. *J.D. Shi, C.Y. Wang, Y.Q. Huang, P.E. Cruz, J.X. She.* Dept Pathology/Immunol/Lab Med, Univ Florida, Gainesville, FL.

The Urofaical (Ochoa) syndrome (UFS) is an autosomal recessive disease. The major characteristics of this syndrome are congenital obstructive uropathy and abnormal facial expression. Our previous study narrowed the gene to two overlapping BAC clones, a region of less than 360 kb genomic DNA. In the present study, we have isolated a novel candidate gene (UFSC1) within the UFS critical region from one of the two BAC clones (369I8). The UFSC1 gene consists of 4.8-kb nucleotide sequence containing an ORF of 1759-bp and encodes a putative polypeptide of 586 amino acids. The gene contains 11 exons. We searched for mutations in two UFS patients (4-3 and 6-3) from Columbia and two patients (30-3 and 41-3) from the US as well as one unaffected mother (31-2). The entire coding region, untranslated regions and intron/exon junctions were amplified from genomic DNA using 18 primer pairs and sequenced by an ABI 310 automated sequencer. Comparison of the sequences from patients with normal control failed to detect any pathogenic mutation. Therefore, UFSC1 does not appear to be the gene responsible for the urofacial syndrome.

Program Nr: 2146 from the 1999 ASHG Annual Meeting

The human survival motor neuron (SMN) genes are developmentally regulated. *L.R. Simard, C.F. Rochette, T. Brun, S. Meilleur.* Centre de Recherche, Hopital Sainte-Justine, Montreal, PQ, Canada.

Spinal muscular atrophy (SMA) is an autosomal recessive lower motor neuron disease and the leading monogenic cause of infant mortality. The critical 5q-SMA region contains an inverted repeat element of about 500-kb. Mutations involving the SMN1 gene in the telomeric element are responsible for childhood-onset SMAs. Analyses of SMN interacting proteins has revealed two functions; one involving the assembly and transport of spliceosomal snRNP complexes, the other involving the maintenance of active pre-mRNA splicing complexes. Despite these advances, it remains unclear why mutations in SMN1 alone cause SMA and why loss of SMN1 specifically affects motor neuron cells. To address this issue, we initiated SMN1 and SMN2 gene expression studies. Previous analyses of the 2 kb region upstream of the translation initiation site did not reveal significant differences in SMN1 and SMN2 promoter activity. We now provide evidence that the SMN genes are developmentally regulated. In this study, we have characterized the transcription initiation site(s) of the SMN genes using RNAs prepared from various fetal and adult tissues. Primer extension studies mapped two major initiation sites; one or the other is preferentially used during development. The major SMN transcript in fetal tissues contains a longer 5' UTR than that found in adult tissues, the initiation site mapping 320 nucleotides upstream of the translation start site. This site is used by the single SMN1 gene found in the chimpanzee and transcripts harboring the longer 5' UTR are absent in RNA samples from type I SMA patients suggesting that these transcripts are the primary product of the SMN1 gene. Corroborating 5' RACE and RNase protection studies will be presented. Finally, transient transfection studies in P19 cells indicated that the SMN promoters are 10 fold more active in undifferentiated cells compared to P19 cells treated with either DMSO or retinoic acid. Taken together, these results provide strong evidence that the SMN genes are developmentally regulated and suggest that important differences between SMN1 and SMN2 gene expression may exist. *Funded by MRC and MDAC.*.

Program Nr: 2147 from the 1999 ASHG Annual Meeting

A protein which specifically binds to triplet repeat $(CTG)_n$. *P.A. Slominsky, M.I. Shadrina, S.A. Limborska.* Dept Human Molec Genetics, Inst Molecular Genetics, Moscow, Russia.

In a basis of a dynamic mutation the phenomenon of increase of number of copies (expansion) simple di- and three nucleotide microsatellite repeats lays. Mechanisms and the reasons of expansion are investigated not enough and probably, that the process of expansion is adjusted with participation of specific DNA-protein interactions between repeats and replication complex proteins. For revealing specific $(CTG)_n$ microsatellite binding proteins a method of gel retardation was used. Was shown, that in nuclear and total cell extracts from rat liver and brain are present two $(CTG)_9$ repeat binding proteins. One of these proteins is highly specific - the complex formation did not blocked by 100 fold excess of "cold" unlabelled random oligonucleotide of the same length, but is suppressed significantly already by 10 fold excess unlabelled. cDNA for $(CTG)_n$ microsatellite binding protein was cloned by screening of expressed human fetal brain cDNA with $(CTG)_9$ probe. After cloning and sequencing one of $(CTG)_n$ binding protein appear identical to well known protein, growth/differentiation factor 1 or LAG1. This protein are highly homologous to C.elegans longevity gene. It is possible that $(CTG)_n$ repeat binding protein - such a LAG1 - plays a role in prevention of hairpin formation or hairpin stabilisation and by that suppresses process of triplet repeat expansion. It may be critical for cell surveillance and fate.

Program Nr: 2148 from the 1999 ASHG Annual Meeting

Identification of a Matrix Attachment Region at the X-linked Transferrin Locus. *P. Smith, D. Catalaa, M.A. Wong, W. Zupko, M.A. Goldman.* Dept Biol, San Francisco State Univ, San Francisco, CA.

The transferrin transgene array consists of 11 tandem repeats of a 17 kb construct which includes the 11 kb transferrin gene as well as 6 kb of 5' and 3' flanking sequence. In contrast to most X-linked genes, transferrin is expressed from both the active and inactive X chromosomes. It is suggested that transferrin constitutes its own chromatin domain(s), allowing it to maintain a transcriptionally competent conformation amidst an otherwise transcriptionally silent environment. A nuclear matrix binding assay was employed in an attempt to locate matrix attachment regions (MARs), which are sequence elements that preferentially bind to the nuclear matrix, and which serve as endpoints of chromatin domains. A 1.6 kb fragment consisting of roughly equal parts of the 3' end of transferrin and the 3' flanking region was identified as a MAR. Significantly, this fragment contains a topoisomerase II recognition sequence, which is a consistent feature of MARs. Also, the location of the MAR coincides with the location predicted by the MARfinder algorithm. Further cloning of this region should facilitate more precise mapping of the MAR. Also, we predict the presence of a MAR in the 5' region of transferrin.

This work was supported in part by an NIH Academic Research Enhancement Award, an NIH Minority Biomedical Research Support grant, and an NIH Research Infrastructure for Minority Institutions grant.

Program Nr: 2149 from the 1999 ASHG Annual Meeting

Inhibition of FMR1 gene expression with phosphorothioate antisense oligonucleotides. *O. Sobolev[1], PJ. Hagerman[2], MB. Brennan[1], F. Tassone[2].* 1) Eleanor Roosevelt Institute, Denver, CO; 2) Department of Biochemistry and Molecular Genetics,UCHSC, Denver, CO.

FMRP is an RNA-binding protein containing both nuclear localization and nuclear export signals. FMRP binds to its own message with high affinity and interacts with a number of other human fetal brain messages. It is important to identify the RNA species within the cell to which FMRP binds, because it appears likely that loss of binding to such RNAs might result in the pleiotropic phenotype associated with fragile X syndrome. Furthermore, it is not clear how FMRP binding influences stability, localization and translation efficiency of this class of RNAs. One of the major problems in identifying changes in mRNA levels in the absence of FMRP is the isolation of FMRP-specific effects in RNA backgrounds that may differ between different cell lines. Decreasing FMR1 gene expression may be important for identifying the mRNAs to which FMRP binds. To circumvent this problem, we have tested phosphorothioate antisense oligodeoxynucleotides (PS-ODNs) targeted against the FMR1 gene to specifically disrupt its expression. We have used a number of PS-ODNs on human cultured fibroblasts. Cells were treated in the presence of a lipofecting reagent for 5 days. Total RNA was then isolated, reverse transcribed, and the amount of FMR1 cDNA was quantified by fluorescence RT-PCR. Two of these antisense PS-ODNs resulted in a significant decrease in FMR1 mRNA expression level (up to 90%). Differentially expressed mRNAs are currently being identified using cDNA arrays.Results will be discussed.

Program Nr: 2150 from the 1999 ASHG Annual Meeting

Clonning and mapping of human FTCD, a candidate for the glutamate formiminotransferase deficiency (FIGLU-URIA). *A. Solans, X. Estivill, S. de la Luna.* Medical and Molecular Genetics, IRO, Barcelona, Spain.

Human chromosome 21 (HC21) has been intensively studied, mainly because trisomy 21 causes Down syndrome (DS) and because it is the smallest human chromosome, thus acting as a model for studies on other human chromosomes. HC21 is estimated to contain 300-400 genes (EMBO Workshop Molecular Biology of Chromosome 21 and Down Syndrome, June 1999). International gene cloning efforts have led to the identification of numerous genes and transcriptional units and more recently, large scale sequencing has enabled the prediction of many more. Nowadays 112 genes (GDB, June 13, 1999) have been assigned to HC21. However, the function of most of these genes remains unknown. In order to contribute to the development of the transcriptional map of human chromosome 21, we have used in silico cDNA cloning by searching NCBI databases (UNIGENE, GeneMap'98). In these searches a novel human gene, hFTCD, has been identified by its sequence similarity to porcine FTCD (Formiminotransferase ciclodeaminase, EC_number=2.1.2.5, EC_number=4.3.1.4), an intermediate metabolism enzyme involved in the histidine degradation pathway. hFTCD full length cDNA has been cloned and mapped to 21q22.3. The gene is expressed, as different mRNA species, mainly in adult and foetal liver; expression can also be detected in adult kidney and testis, and in foetal tissues such as kidney and lung. The human FTCD amino acid sequence shows a high degree of identity to a putative protein from Dictyostelium discoideum and predicted ORFs in prokaryotic organisms including Streptococcus pyogenes, Porphiromonas gingivalis, Thermotoga maritima, Chlorobium tepidum and Clostridium acetobutylicum. In some of these, the FTCD amino acid sequence seems to be split into two separate ORFs corresponding to the two different activities of FTCD, and thus suggesting that the eukaryotic gene may have evolved by fusion of two distinct prokaryotic cistrons. FIGLU-URIA (OMIM 229100) has been described as a glutamate formiminotransferase deficiency. Searching of mutations in hFTCD will allow to study the implication of this new described gene in the disease.

Program Nr: 2151 from the 1999 ASHG Annual Meeting

TRPC5, the human homologue of a mouse brain transient receptor potential Ca^{2+} channel. *K. Sossey-Alaoui, J.A. Lyon, L. Jones, F.E. Abidi, A.J. Hartung, B. Hane, C.E. Schwartz, R.E. Stevenson, A.K. Srivastava.* Center for Molecular Studies, J. C. Self Research Institute of Human Genetics, Greenwood Genetic Center, Greenwood, SC.

A novel human gene, TRPC5, was cloned from the region of Xq23 that contains loci for non-syndromic mental retardation (MRX47 and MRX35) and two genes, DCX and HPAK3, implicated in two X-linked disorders (LISX and MRX30). Within a single YAC, we have also mapped a placenta-specific gene that encodes a calpin-like protein, CAPN6, and determined the order, cen - HPAK3 (5'-3') - CAPN6 (3'-5') - DCX (3'-5') - DXS7012E - TRPC5 (3'-5')/DXS1059 - ter. Here we report the detailed characterization of the most telomeric gene, which encodes a 974 residue novel human protein, TRPC5 (111.5 kDa predicted mass) and displays 99% homology with mouse trp5, a novel member of a family of receptor-activated Ca^{2+} channels. It contains eight transmembrane domains, including a putative pore region. A transcript larger than 9.5 kb is observed only in fetal and adult human brain, with a relatively higher level in the adult human cerebellum. A 4.0 kb transcript variant is detected in pancreas. The human TRPC5 gene comprises 11 exons spanning about 300 kb of genomic DNA.

We devised an efficient method, Incorporation PCR SSCP (IPS), for detection of gene alterations. Five single nucleotide variations in the TRPC5 gene were identified in males with mental retardation. However, these were found to be polymorphic variants. Exclusive expression of the TRPC5 gene in developing and adult brain suggests a possible role during development and provides a candidate gene for instances of mental retardation and other developmental defects.

Program Nr: 2152 from the 1999 ASHG Annual Meeting

Identification of novel alternatively spliced human biotinidase mRNAs that potentially encode distinct signal peptides and are expressed in a tissue specific manner. *C.M. Stanley, B. Wolf.* Department of Human Genetics, Medical College of Virginia of Virginia Commonwealth University, Richmond, VA.

Biotinidase is an enzyme essential for biotin recycling. Children with an inherited deficiency of biotinidase have been effectively treated with biotin. Biotinidase has been suspected of having multiple functions possibly requiring it to be located in different cellular and extracellular spaces. To better understand more about this process, we have identified and characterized three human biotinidase mRNA splice variants using rapid amplification of cDNA 5' ends-polymerase chain reaction (5'-RACE-PCR). Sequence analysis of the amplified products resulted in additional 5'sequence, extending the former exon 1, now called 1a, and two novel alternative exons, 1b and 1c. These three distinct transcripts appear to have resulted from alternative splicing of exons 1a, 1b and 1c at the 5' end of the biotinidase pre-mRNA. Northern blot analysis was used to determine the tissue-specific expression of each splice variant. The 1a and 1b splice variants were identified in various human tissues including liver, kidney, pancreas, placenta, testes, whole brain and caudate nucleus, but the proportion of expression differed in each tissue type. Exon 1a was the most highly expressed variant in liver, whole brain and caudate nucleus while exon 1b was a minor variant in these tissues. Both 1a and 1b were expressed in equal proportions in pancreas and placenta. Exons 1a and 1b each contained two putative, in-frame, AUG, translation initiation codons, that are predicted to result in distinct signal peptides. The 1c splice variant was found only in testes, and its expression in testes was confirmed by both 5'-RACE-PCR and a published expressed sequence tag. Moreover, exon 1c was the most abundant variant in testes and contained three in-frame AUGs, but also contained an in-frame stop codon (TGA) at its 3' end. Depending on the site of translation, each splice variant could encode a distinct signal peptide, which may result in tissue-specific intracellular localization of biotinidase. Further studies are examining the intracellular localization of biotinidase in these various tissues.

Program Nr: 2153 from the 1999 ASHG Annual Meeting

Genomic structure and localization within Y chromosome interval 6 of the BPY2 gene. *L. Stuppia[1,2], V. Gatta[1], I. Fogh[3], G. Calabrese[1,4], D. Fantasia[1,4], F. Capodiferro[1,4], E. Morizio[1,4], A. Ratti[3], A. Pizzuti[3,5], G. Palka[1,4].* 1) Dept di Science/Biomed, Univ G D Annunzio, Chieti, Italy; 2) Istituto di Citomorfologia Umana CNR, Chieti, Italy; 3) Istituto di Clinica Neurologica, Univ. di Milano, Italy; 4) Servizio di Genetica Umana, Ospedale Civile di Pescara, Italy; 5) C.S.S. - Mendel Institute, Roma, Italy.

In recent years, a number of studies have investigated the relationship between male infertility and Yq microdeletions, which are detected in about 10% of infertile patients. So far, at least 12 genes have been isolated within Yq, in the regions involved by the microdeletions, but only RBM1, DAZ and DFFRY genes have been specifically analyzed as candidate for male infertility. In this study, we investigated BPY2 (Basic Protein 2) gene, which has been mapped within the subinterval 6E of the Y chromosome, where the presence of a locus specific for oligozoospermia have been previously suggested. PCR analysis with specific primers for BPY2 performed on 7 patients with previously detected microdeletions of subinterval 6E showed the presence of a normal amplification product, thus suggesting a different localization of the gene or the presence of multiple copies. In order to characterize BPY2, we performed a screening of a human Y chromosome YAC library, and identified the clone yOX10 as containing BPY2. Cosmid clones were derived, and sequencing of the clone 26 allowed to indentify the genomic structure of BPY2, which consists of 8 exons, with the first ATG codon located within exon 4. Cosmid 26 was used as a probe for two colors fiber FISH analysis, which displayed two copies of BPY2, one localized within the DAZ gene cluster, and the second one mapped within subinterval 6E. These results indicate that deletions of DAZ cluster should cause the loss of at least one copy of BPY2 gene, suggesting that the role of this gene in male infertility should be evaluated. Further studies are in progress in order to investigate the presence of BPY2 deletions or mutations in infertile patients.

Program Nr: 2154 from the 1999 ASHG Annual Meeting

HMG20A and HMG20B map to human chromosomes 15q24 and 19p13.3 and constitute a distinct class of HMG-box genes with ubiquitous expression*HMG20A HMG20B*. *L. Sumoy[1], L. Carim[1], M. Escarceller[1], M. Nadal[1], M. Gratacos[1], M.A. Pujana[1], X. Estivill[1], B. Peral[1,2].* 1) Dept. de Genètica Molecular, Institut de Recerca Oncològica, Hospital Duran i Reynals, L'Hospitalet, Barcelona, Spain; 2) Unidad de Neurología, Fundación Jiménez Díaz, Madrid, Spain.

Working within the EURO-IMAGE Consortium full-length cDNA sequencing initiative, we have discovered HMG20A and HMG20B, two novel human HMG box-containing genes. The HMG box encodes a conserved DNA binding domain found in many proteins with roles in the regulation of transcription and chromatin conformation. The predicted HMG20A and HMG20B proteins encoded by these two genes are 48% identical (74% within the HMG domain). The HMG domain of both HMG20 proteins is most similar to that of yeast NHP6A (38-42%), human BAF57 (33-38% identity; 58-59% similarity to HMG20A-HMG20B respectively) and human SOX14 (32-36% identity; 58-59% similarity to HMG20A-HMG20B respectively). Outside of the HMG domain, HMG20 proteins lack any significant homology to other known proteins. Phylogenetic and protein comparison analysis excludes HMG20A and HMG20B from the TCF/SOX group of HMG proteins, and places them along with HMG1/2 type proteins which bind to DNA in a non-sequence specific fashion. However, HMG20 genes do not branch together with any of the established HMG1/2 subtypes. We determined the genomic structure and expression pattern of HMG20A and HMG20B. Both genes have several alternative transcripts, expressed almost ubiquitously. mRNA sizes are 1.5, 4 and 9 kb for HMG20A, and 1.5 and 2.6 kb for HMG20B. HMG20A maps to human chromosome 15q24 (D15S1227) and HMG20B to 19p13.3 (D19S209-D19S216, between TBXA2R and CTF-1). Five other gene pairs map to these two regions indicating the possible existence of homologous gene clusters in 15q24 and 19p13.3. We isolated mouse Hmg20A and Hmg20B cDNAs and found very strong identity between the human and mouse counterparts. Thus, HMG20 genes define a distinct class of mammalian HMG-box genes. Funded by EU Biomed Project BMH4-CT97-2284.

Program Nr: 2155 from the 1999 ASHG Annual Meeting

Amino acid homologies between human biotinidase and bacterial aliphatic amidases: Putative identification of the active site of biotinidase. *K.L. Swango[1], J. Hymes[1], P. Brown[4], B. Wolf[1,2,3]*. 1) Department of Human Genetics; 2) Department of Pediatrics; 3) Department of Biochemistry and Molecular Biophysics, Medical College of Virginia of Virginia Commonwealth University, Richmond, Virginia 23298; 4) Molecular Biology and Biophysics Section, Division of Biomedical Sciences, King's College London, Strand, London WC2R 2LS, United Kingdom.

Biotinidase is an aminohydrolase that cleaves biocytin, the degradation product of carboxylase turnover, to regenerate the vitamin biotin. Biotinidase deficiency is an inherited disorder characterized by neurological and cutaneous symptoms. The cutaneous symptoms can be effectively treated with the administration of oral biotin. If diagnosis is made at birth and biotin treatment is begun immediately, all symptoms can be prevented. A search of protein databases revealed amino acid homologies for human biotinidase, bacterial aliphatic amidases, and some bacterial and plant nitrilases. Previous studies have shown that amino acid sequences of aliphatic amidases and nitrilases contain four major regions of homology, one of which contains the cysteine involved in the active site of both enzymes. Human biotinidase has considerable homology to these enzymes, particularly in this latter region. This suggests that Cys_{245} is likely the cysteine in the active site of biotinidase and the site of the putative thioester formation that is integral for enzyme function. In addition, because the slightly upstream sequence, $YRK_{210\text{-}212}$, is highly conserved among the three enzyme families and because there are naturally-occurring mutations that cause profound biotinidase deficiency within this region, this region is likely to be essential for enzyme activity. Aliphatic amidase from *Pseudomonas aeruginosa* does not have biotinyl-hydrolase or biotinyl-transferase activities, probably because it lacks a biotin-binding site. In addition, purified human biotinidase does not cleave the substrate used to assay aliphatic amidase activity. Homology between biotinidase and the aliphatic amidases putatively localizes the active site of biotinidase and suggests that the active site of biotinidase is conserved from bacteria.

Program Nr: 2156 from the 1999 ASHG Annual Meeting

Localization of MYOC (GLC1A) transcripts in human eye and optic nerve by in situ hybridization. *R.E. Swiderski[1], J.H. Fingert[2], J.L. Ross[3], W.L.M. Alward[2], G.S. Hageman[2], E.M. Stone[2], V.C. Sheffield[1,4].* 1) Dept Pediatrics, Univ Iowa, Iowa City, IA; 2) Dept of Ophthalmology; 3) Central Microscopy Research Facility; 4) The Howard Hughes Medical Institute.

The glaucomas are a heterogeneous group of diseases that are unified by characteristic optic nerve head damage with associated loss of peripheral vision. Primary open angle glaucoma (POAG) is the most commmon form of glaucoma, affecting 1-2% of the population over the age of 40. Recently, our laboratory demonstrated that mutations in the human MYOC (GLC1A) gene are associated with juvenile open angle glaucoma (JOAG) as well as typcial late-onset POAG. Examination of dissected human ocular tissues or derived cell lines by Northern blotting as well as in situ hybridization analysis of mouse eyes indicates that MYOC is widely expressed in ocular tissues. In this study, we confirmed these findings using in situ hybridization analysis of human eye tissue and extended the study to include the optic nerve which is the primary site of neuropathy in the disease. In the anterior region of the eye, MYOC transcripts were present in the corneal stroma, beneath the anterior surface of the iris and in the iris stroma, in the ciliary muscle, and in the sclera. Elevated expression was noted in the trabecular meshwork, a region of the anterior segment associated with the elevated intraocular pressure frequently observed in POAG. MYOC expression in the optic nerve was localized to the dura mater, arachnoid, subarachnoid trabeculae, pia mater, perivascular connective tissue surrounding the central retinal vessels, and in the fascicles of optic nerve fibers. The widespread MYOC expression pattern suggests that an important role for the cognate protein, myocilin, in the structure and function of the eye.

Program Nr: 2157 from the 1999 ASHG Annual Meeting

Investigating the influence of sequence diversity in the *CDKN2A* gene on its expression and melanoma susceptibility. *T.-L. Tseng[1], A.M. Goldstein[2], J.P. Struewing[1]*. 1) Laboratory of Population Genetics &; 2) Genetic Epidemiology Branch, NCI, Bethesda, MD.

Single nucleotide polymorphisms (SNPs) are common within and near the coding regions of cancer susceptibility genes, including the *CDKN2A* gene. These SNPs alone, however, are not often highly predictive of cancer susceptibility. This may be due to the diversity of gene expression, such as splicing diversity due to SNPs within exonic splicing regulatory elements. We have systematically examined the expression patterns of p16 in more than 40 melanoma and fibroblast cell lines carrying alterations in *CDKN2A*. Surprisingly, we detected smaller proteins in certain cell lines, ranging between 8 kDa and 15 kDa, by western blotting with p16 antibodies. Because of the cell line specific patterns, we believe that these smaller proteins are either the products of aberrant splicing or sequence dependent proteolysis but not degraded p16 due to the extraction procedures. From a cell line carrying a splice site mutation, we have identified and cloned a splicing variant that has not been described previously. This novel alternative splicing variant skips the entire exon 2 and conceptionally translates 89 amino acids. Interestingly, we have also observed an aberrant protein close to 9.4 kDa, p9, in this cell line. Based upon the close correlation in the expression pattern between RT-PCR and western blotting, we believe that this is not a rare aberrantly splicing variant of p16 amplified by RT-PCR but a physiological splicing variant that can express a novel stable protein *in vivo*. Moreover, a low level of this alternative splicing product can be detected by exon1 and 3 junction specific primers in several cell lines carrying different single nucleotide alterations. We are currently investigating the relationship between the expression of p9 and the development of melanoma, as well as identifying the environmental factors that may affect the expression levels of aberrant splicing in cell lines carrying SNPs in the *CDKN2A* gene. This study will offer an excellent model system for understanding how SNPs in genes may influence disease susceptibility via splicing diversity.

Program Nr: 2158 from the 1999 ASHG Annual Meeting

The role of p80 coilin in murine development and coiled body formation. *K.E. Tucker[1], E.Y. Jacobs[1], E.K. Chan[2], L. Massello[1], D.F. LePage[1], R. Conlon[1], A.G. Matera[1].* 1) Department of Genetics, Case Western Reserve University, Cleveland, OH; 2) W. M. Keck Autoimmune Disease Center, La Jolla, CA.

Coiled bodies (CBs) are conserved nuclear organelles that have been identified in numerous species from plants to *Drosophila*, *Xenopus*, and humans. Although the function(s) of CBs are poorly understood, they have been implicated in many nuclear processes, including snRNP biogenesis, snRNA transcriptional regulation, and histone RNA maturation. CBs contain many nuclear epitopes including fibrillarin, Nopp140, the splicing snRNAs, the survivor motor neuron protein (SMN), and the coiled body marker protein p80 coilin. To gain insight into the role of coilin in CBs, the nucleus, and the organism as a whole, we have generated a knockout mouse deleted for exons 2 through the coilin stop codon at the 5 end of exon 7.

Northern and western analyses demonstrate that we have successfully removed the coilin protein from our knockout animals. Preliminary studies of the animals reveal that mice lacking the coilin protein are both viable and fertile and cannot be distinguished phenotypically from their heterozygote and wildtype littermates. We have also derived mouse embryonic fibroblasts (MEFs) from coilin null embryos. These cells will be used to determine the presence, composition and behavior of CBs in the absence of the coilin protein. MEFs will also be used to examine nuclear and nucleolar architecture in the presence of mutant coilin proteins. In order to better understand the role of coilin in CB formation, transient transfection experiments using various coilin constructs in the null cell lines will also be described.

Program Nr: 2159 from the 1999 ASHG Annual Meeting

Characterisation of the human snail (*SNAI1*) gene and exclusion as a major disease gene in craniosynostosis.
S.R.F. Twigg, A.O.M. Wilkie. Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, UK.

The snail family in vertebrates comprises two zinc-finger transcription factors, snail and slug, that are thought to be involved in the formation of the mesoderm and neural crest. We have isolated and characterised the human snail (*SNAI1*) gene and a related snail-like pseudogene (*SNAI1P*), both homologues of the mouse snail gene (*Sna*). Initially, using a *Sna* cDNA probe, a genomic clone with DNA sequence showing 79.3% identity to *Sna* was isolated. This gene, which mapped to 2q34, contained a substantial open reading frame but no introns. Moreover, the potential coding sequence was preceded by an *Alu* element, encoded only 2 *bona fide* zinc-fingers, and did not yield any detectable cDNA. Collectively, these observations suggested that this sequence represented a non-functional processed pseudogene. We subsequently isolated a second genomic clone that contains *SNAI1*. This gene spans approximately 6.4 kb, contains three exons and has a CpG island upstream of the coding sequence. A single transcript of 1.9 kb was detected in several human fetal tissues, with highest expression in kidney. The *SNAI1* open reading frame encodes a protein of 264 amino acids containing 4 zinc-finger motifs that shows 87.1% identity to *Sna*. FISH and radiation hybrid analysis indicates probable localisation between markers *D20S109* and *D20S196* in 20q13.

It has been postulated that mutations in the human orthologue of snail may result in congenital anomalies, because mutations in *TWIST*, a gene thought to have a complementary role to that of snail in mesoderm formation, cause Saethre-Chotzen syndrome. To address this issue, we have searched for DNA variants within the *SNAI1* gene, in patients with craniosynostosis, by single-strand conformation polymorphism (SSCP) analysis. This excluded *SNAI1* as a major disease gene in craniosynostosis. Two single nucleotide polymorphisms encoding synonymous amino acids were identified in exon 2; these may be helpful for analyses of allele-specific expression and linkage mapping. Our localisation of *SNAI1* to 20q13. leaves open the possibility that it is involved in other congenital or acquired diseases mapping to this region.

Program Nr: 2160 from the 1999 ASHG Annual Meeting

The protein encoded by *FRG1*, a candidate gene for FSHD, is localized in the nucleolus. *S.M. van der Maarel[1], S. van Koningsbruggen[1], G. Deidda[2], R.J.L.F. Lemmers[1], G.W. Padberg[3], J. Hewitt[4], R.R. Frants[1]*. 1) Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands; 2) Institute of Cell Biology, CNR, Rome, Italy; 3) Department of Neurology, University Hospital Nijmegen, Nijmegen, The Netherlands; 4) Institute of Genetics, University of Nottingham, Nottingham, United Kingdom.

Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant myopathy mainly characterized by the progressive wasting of the facial, shoulder and upper arm muscles. The major locus, FSHD1, is mapped to the subtelomere of chromosome 4q. This subtelomere is composed of a polymorphic repeat array consisting of 3.3kb units (D4Z4). In the population, the number of units may vary between 10 and >150, while patients, due to the deletion of an integral number of repeat units, carry an array <10 units.

FSHD is likely caused by a position effect in which the deletion in the repeat array causes the dysregulation of genes nearby. The first gene identified, *FRG1*, is localized 120kb proximal to the repeat array. Several features make this gene an interesting candidate gene for FSHD: the close proximity to the rearrangement, the high conservation between vertebrates and invertebrates, and the possible upregulation of gene expression in cultured myoblasts of patients.

FRG1 encodes a protein of 258 amino acids with unknown function. To obtain more insight in its function, we generated fusion constructs of FRG1 with a FLAG epitope. After transfection, the protein was exclusively detected in the nucleolus where it co-localizes with rDNA. Ongoing EM studies should reveal its subcellular localization. Deletion constructs are currently analyzed to define functional subdomains of the FRG1 protein.

These studies will provide a better understanding of the biological function of FRG1 and may explain its high conservation between different species. Hopefully, it may give us more insight in the putative role of FRG1 in the pathophysiology of FSHD.

Program Nr: 2161 from the 1999 ASHG Annual Meeting

Genomic structure of the human DLX4/7 gene: evidence for alternative splicing . *S.J. Walker[1], J.A. Price[2], J.T. Wright[3], T.C. Hart[1]*. 1) Dept Pediatrics/Med Genetics, Wake Forest University Baptist Medeical Center, Winston-Salem, NC; 2) NIH Bethesda, MD; 3) Dept. Pediatric Dentistry, University North Carolina, Chapel Hill, NC.

The Distal-less (Dll) gene of Drosophilia is a single-copy regulatory gene that specifies a homeobox-containing protein required for normal limb development. In vertebrates there is an entire class of Dll-like homeobox transcription factors, identified by the conserved 60 amino acid (homeodomain) they have in common. In humans, six DLX genes have been identified and localized; DLX1 with DLX2 on chromosome 2q near the HOXD locus; DLX5 and DLX6 on chromosome 7 with the HOXA cluster; and DLX3 with DLX4 or DLX7 on chromosome 17q21, 1-2 Mb from the HOXB cluster. Human DLX7 and DLX4 cDNAs have been previously cloned and sequenced from a leukemia cell line and from a placental cDNA library, respectively. We describe here the subcloning and sequencing of a 3.8 kb Xba1 genomic DNA fragment that contains the entire human DLX4/7 coding region, including several kilobases of 5' and 3' untranslated DNA. This report of the genomic sequence identifies two protein coding regions, separated by a 430 base-pair intron. A comparison of this genomic sequence with the published sequences of human cDNA clones from two different sources (DLX7 from leukemis cells and DLX4 from 32-week placenta) revealed the presence of two different mRNAs which have resulted from alternative splicing. The sequence comparison also revealed that DLX4 and DLX7 are isoforms of the same gene. We have confirmed the presence of both of these species in placental RNA and herein present the genomic organization of DLX4/7. The presence of alternatively spliced RNAs for this homeobox-containing gene suggests a regulatory mechanism that may be tissue-specific.

Program Nr: 2162 from the 1999 ASHG Annual Meeting

Characterization of murine subunit 3 of the COP9 signalosome; genomic structure and expression pattern. *K. Walz, C. Boerkoel, J. Yang, J.R. Lupski.* Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Smith-Magenis syndrome (SMS) is a multiple congenital anomalies mental retardation syndrome associated with a heterozygous deletion of 17 p11.2. Clinical features include neurobehavioral abnormalities and sleep disturbances. One of the genes mapping in the critical deletion interval encodes the subunit 3 of the COP9 signalosome. The COP9 signalosome is a multi-subunit complex conserved between plants and mammals. In *Arabidopsis* this complex functions as a repressor of photomorphogenesis. Considering the function of this complex in plants and its conservation among species, haploinsufficiency of this gene (Cop9S3) may play a role in the disturbed circadian rhythm in SMS patients. In order to generate a knock out mouse for the Cop9S3 gene, we determined its genomic structure. Eight exons have been characterized todate, using cDNA and genomic sequencing. Additionally, a vector has been created which will generate a disruptive integration of 10 kb in the Cop9S3 gene and is being targeted in ES cells. Northen blot analysis using the whole cDNA as a probe detected a 1.8 kb transcript expressed ubiquitously in mouse, with strongest hybridization to heart, brain, liver, kidney and testis tissues. Some larger mRNAs of 4-6 kb are also observed in all tissues examined. The expression pattern in humans is quite similar suggesting that the knock out mouse will be an invaluable tool to undestand the contribution of Cop9S3 to the SMS phenotype.

Program Nr: 2163 from the 1999 ASHG Annual Meeting

Identification, mapping and genomic structure of a novel X-chromosomal human gene (SMPX) encoding a small muscular protein. *M.S. Wehnert[1], D. Patzak1[1], O. Zhuchenko[2], C.C. Lee[2]*. 1) Inst Human Genetics, Ernst-Moritz-Arndt Univ, Greifswald, Germany; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, One Baylor Plaza, Texas 77030, USA.

By reciprocal probing, a cDNA clone (xh8H11) was identified representing a gene preferentially expressed in striated muscle. The gene maps close to DXS7101 31.9 cM from the short arm telomere of the X-chromosome at Xp22.1. Searching expressed and genomic databases, 21 ESTs were found that allowed to assign a human extended consensus sequence of 886 bp suggesting a completely expressed gene symbolized as SMPX. Using the human consensus sequence, the orthologous mouse Smpx and rat SMPX genes could be aligned. An open reading frame was identified encoding a peptide of 88 to 86 and 85 amino acids in human and rodents, respectively. The predicted peptide has no significant homologies to known structural elements. BLASTN searches allowed to define the genomic structure of SMPX which consists of five exons (172, 57, 84, 148, 422 bp) and four introns (3639, 10410, 6052, 31134 bp) comprising together 52.1 kb. The gene is preferentially and abundantly expressed in heart and skeletal muscle. Thus a novel human gene encoding a Small Muscular Protein that maps to Xp22.1 (SMPX) has been identified and structurally characterized.

Program Nr: 2164 from the 1999 ASHG Annual Meeting

Changing concepts of the gene. *J.A. White, E.A. Bruford, H.M. Wain, M.S. Povey.* Galton Laboratory, Univ Col London, London, UK.

There is increasing evidence that even in the mammalian genome a single stretch of DNA can be utilised in several different ways. Fascinating as this is, it does pose some problems for nomenclature. The "gene" remains a useful concept and clearly a working definition is needed. The current definition used by the HUGO gene nomenclature committee is deliberately somewhat vague: *A gene is a DNA segment that contributes to phenotype/function.*

Most genes are easily recognized as such, but a significant proportion challenge our current definition. We present a few examples.

1. Fusion genes: in addition to the products of individual genes, fusion transcripts are produced from segments of a number of different loci along the same DNA strand. eg *HHLA1*, *OC90* and the HERV-H element (Genomics 57:371-9).

2. Alternative reading frames: transcripts from different frames at the same locus. eg. p16(INK4a) and p14(ARF) from the *CDKN2A* locus (EMBO J 17:5001-14).

3. Bicistronic genes: two products from adjacent or overlapping stretches of the same mRNA eg *SNRPN* and *SNURF* (PNAS USA 96:5616-21).

4. Antisense genes: a gene product encoded on the anti-sense strand of another gene eg *ORCTL2* and *ORCTL2S* (Genomics 49:38-51).

5. Imprinted genes: product depends on parental origin of the allele, many unusual mRNA examples (bicistronic, antisense, non-coding) are also imprinted. eg An imprinted antisense transcript - LIT1- within the *KCNQ1* locus (Hum Mol Genet 8:1209-1217).

These situations may be interpreted differently, but decisions are required about which entities are assigned approved gene symbols. It is becoming ever more important to be aware of the different levels of nomenclature for loci, genes, transcripts and proteins (as well as phenotypes, mutations, alleles etc). This requires increased communication and collaboration between different nomenclature groups, as well as appropriate database structures to represent the relationships. A consensus among scientists about the philosophy would however be a start, and we welcome comments.

Program Nr: 2165 from the 1999 ASHG Annual Meeting

Expression of the human 21-hydroxylase (CYP21) gene requires far upstream elements within the C4B gene: the evolutionary drive to maintain two unrelated genes in close linkage. *S.D. Wijesuriya, A. Dardis, G. Zhang, W.L. Miller.* Pediatrics, UCSF, San Francisco, CA.

Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase (P450c21) deficiency is caused by mutations in the CYP21 gene and occurs in 1 in 14000 individuals leading to errors in sexual differentiation and potentially lethal salt loss. P450c21 is expressed only in the adrenal cortex. The 5' ends of the human CYP21 genes lie 2466 bp downstream from the complement component C4 genes. 300 bp of the proximal promoter and two regions between exons 38 and 40 of the mouse slp C4 gene are known to be required for expression of the mouse CYP21A gene and we have identified a cryptic adrenal-specific Z promoter within intron 35 of human C4. To investigate the upstream regulatory elements affecting the human CYP21 gene, the 5' flanking region up to -9 kb from the CYP21 transcription initiation site was examined using promoter/reporter constructs and transient transfection assays in human adrenal NCI-H295A cells. The 300 bp proximal promoter provided substantial transcriptional activity, but addition of DNA to -4.6 kb, including the regions that activiate mouse CYP21, did not increase activity. Constructs retaining the Z promoter DNA at -4.6 to -5.6 kb, with or without the DNA from -0.3 to -4.6 kb, had 2-3 fold increased promoter activity indicating the presence of distal *cis* regulatory elements. DNase-I footprinting identified two protein binding sites (F1 and F2) lying -132 and -179 bp upstream of the Z cap site (-4.8 and -5.0 kb upstream of the CYP21 cap site). Band shift assays confirmed binding specificity and identified specific protein DNA complexes with each element. Mutations in these elements abolished complex formation and decreased reporter expression in NCI-H295A cells. Oligonucleotides corresponding to the wildtype but not mutant regions conferred expression to a heterologous TK32 promoter. Competetive gel mobility assays identified the F2 site as binding SF-1, but the factor binding the F1 site is unknown. These data indicate that the Z promoter or elements therein are far upstream components of the C21 promoter needed to drive its expression.

Program Nr: 2166 from the 1999 ASHG Annual Meeting

Genomic characterization of the SMN interacting protein (SIP1) and molecular genetic analysis of patients with spinal muscular atrophy. *B. Wirth[1], C. Helmken[1], A. Wetter[1], F. Schoenen[1], T. Liehr[2], S. Rudnik-Schöneborn[1,3], K. Zerres[1,3]*. 1) Inst. Human Genetics, Bonn, Germany; 2) Inst. Human Genetics and Anthropology, Jena, Germany; 3) Inst. Human Genetics, Aachen, Germany.

Autosomal recessive spinal muscular atrophy (SMA) is caused by mutations within the telomeric copy of the survival motor neuron gene (SMN1) on 5q13. The molecular genetic analysis of 525 type I-III SMA patients revealed homozygous mutations of SMN1 in 96%, while 4% failed to show any mutation, despite a typical SMA phenotype (Wirth et al. 1999, Am J Hum Genet 64:1340-1356). Additionally, we have shown that sibs with homozygous absence of SMN1 and identical SMN2 copy number, can show variable phenotypes which implies that SMA is modified by others, yet unknown factors. We therefore analysed the SMN interacting protein (SIP1) which belongs to the same large protein complex of 300 kD as SMN. The SMN-SIP1 complex has an essential function in snRNP biogenesis and both proteins are localized in so called gems within the nucleus. First, we identified the genomic location of SIP1 and assigned it to chromosome 14q13-q21 by fluorescence in situ hybridization of a BAC clone that contains the entire gene. No similar disorder has yet been assigned to this chromosomal region. Furthermore, we determined the exon-intron structure of the SIP1 gene which allowed us to analyse those SMA patients from whom no RNA was available. We sequenced either RT-PCR products or genomic DNA covering the complete coding region from 20 SMA patients, who failed to show any SMN1 mutation. Unfortunately, no mutation has been found within SIP1. Additionally, we sequenced RT-PCR products of the entire SIP1 coding region from sibs of SMA families with identical genotype but different phenotype and again no mutation was found. Finally, we identified five SIP1 transcription isoforms caused by alternative splicing. Quantitative analysis of SIP1 transcription products in SMA patients and controls is in progress. Based on the data obtained so far, we were able to show that neither the phenotypic variability nor the 5q-unlinked SMAs are caused by mutations within SIP1.

Program Nr: 2167 from the 1999 ASHG Annual Meeting

***JAK3* genomic sequence in human B-cell precursors.** *C.M. Wood, F.M. Uckun.* Molecular Genetics, Parker Hughes Cancer Ctr/HI, St Paul, MN.

Janus tyrosine kinase 3 (JAK3) is one of a number of key regulatory enzymes in human B-cell precursors. The gene for *JAK3* has been mapped to chromosome 19p12-13.1 and encompasses at least 23 exons. Constitutively high levels of JAK3 activity may contribute to drug resistance and enhanced clonogenicity of leukemic B-cell precursors from children with acute lymphoblastic leukemia (ALL). As part of a systematic effort to determine the genomic sequence of the *JAK3* gene in normal and leukemic B-cell precursors, we sequenced a relatively short region of *JAK3* spanning two introns, previously termed introns 10 and 11. Unexpectedly, a gap was identified in the original *JAK3* genomic sequence within intron 10 across an Alu repetitive element. Furthermore, the sequence we obtained from intron 11 did not match to that previously reported, and the length of the intron was much larger than expected (1.1 kb vs. 414 bp). Four regions with homology to repetitive elements (Alu, 698 bp total, and LINE2, 188 bp total) were seen across the entire sequence covering exons 10-12 (2.1 kb total). Two potential single nucleotide polymorphisms (SNPs) were observed in intron 11. No germline mutation was found across this region in leukemic B-cell precursors from any of the ALL patients examined. Our results significantly extend previous efforts to determine the genomic sequence of *JAK3*.

Program Nr: 2168 from the 1999 ASHG Annual Meeting

A new paramyotonia congenita SCN4A mutation in domain I-S5 of the sodium channel suggests novel regions involved in channel inactivation. *F.F. Wu[1], E. Pegoraro[2], P. Colleselli[3], E.P. Hoffman[1,4].* 1) Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA; 2) Department of Neurological & Psychiatric Sciences, University of Padova, Italy; 3) Unita' Locale Socio-Sanitaria Belluno, Italy; 4) Research Center for Genetic Medicine Washington DC.

Paramyotonia congenita (PC)is a non-progressive muscle disorder showing cold-induced myotonia and transient weakness. A series of missense mutations have been identified in the adult skeletal muscle sodium channel gene (SCN4A). Here we describe a new three generation Italian family segregating PC. SSCP was used to screen all 24 exons of the SCN4A gene. A C to G transversion was observed at nucleotide 796, causing a missense mutation (L266V). This change adds only a single methylene group to the 1,800 aa protein, but is likely causative of PC as the L266 residue is completely conserved throughout evolution, was not observed in 130 control chromosomes, and co-segregated with the disease. Previously characterized missense mutations causing PC have been found to be clustered in Domain IV, particularly in the intracellular loops known to be involved in fast inactivation of the channel. Additional mutations in the extracellular loops of Domain IV have also been found to disrupt inactivation, and these human mutations have led to the identification of novel domains involved in channel inactivation. The novel L266V mutation we have identified is only the second to be found in Domain I, and the first to be seen in the transmembrane S5. The previously published domain I (V445M) family showed an unusual phenotype of painful myotonia, particularly in the intercostal muscles (Ann Neurol 42: 811-14, 1997). Our Italian family shows classic paramyotonia congenita. The localization of this mutation is highly unexpected, and identifies a new region of the channel which was not previously thought to be important for inactivation. Electrophysiological tests of this mutation in eukaryotic expression systems are currently underway.

Program Nr: 2169 from the 1999 ASHG Annual Meeting

Refinement of the genomic structure of *STX1A* and mutation analysis in nondeletion Williams syndrome patients. *Y.Q. Wu[1], L.R. Osborne[2], L.C. Tsui[2], L.G. Shaffer[1]*. 1) Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Genetics and Genomic Biology, the Hospital for Sick Children, Toronto, Ontario, Canada.

Williams syndrome (WS) is a neurodevelopmental disorder characterized by mental disability with unique cognitive and personality profiles, distinctive facial features, vascular anomalies, connective tissue abnormalities, and infantile hypercalcemia. Our previous studies have shown that ~ 90% of WS patients have a consistent-sized deletion involving multiple genes on chromosome 7q11.23. In this study, we identified 5 individuals who met the clinical criteria for WS by history and physical findings; however, molecular analyses using 10 polymorphic markers within the common deletion region, and FISH analyses using probes to the *ELN*, *LIMK1* and *STX1A* loci showed no microdeletions. Since *STX1A* is a nervous system-specific protein implicated in the docking of synaptic vesicles with the presynaptic plasma membrane, *STX1A* is a good candidate for cognitive or behavioral abnormalities found in WS. In this study, the complete genomic structure of the human *STX1A* was elucidated. In our analysis, published exons 3 and 4 were each found to be comprised of two exons. Therefore, exons 3 and 4 have now been assigned as exons 3 and 4, and 5 and 6, respectively. Thus, *STX1A* is composed of at least 9 exons. Knowledge of the genomic structure has allowed us to do mutation screening using the genomic DNA of the 5 WS patients who do not demonstrate deletions. By sequencing the PCR products for each exon, no point mutations were found in these 5 WS patients. Therefore, these results do not support a role of *STX1A* in the WS phenotype; although continued mutation analysis, such as in the promoter region or in other nondeletion patients, may help to elucidate the role of *STX1A* in Williams syndrome.

Program Nr: 2170 from the 1999 ASHG Annual Meeting

PHR1, encodes a pleckstrin homology domain protein expressed at high levels in mammalian photorecepters. *S. Xu[1], R. Ladak[2], D.A. Swanson[1], H. Sun[3], L. Ploder[2], R.R. McInnes[2], D. Valle[1]*. 1) Institute of Genetic Medicine and HHMI, Johns Hopkins Univ, Baltimore, MD, 21205; 2) Department of Genetics, Hospital for Sick Children, Toronto, Ontario, CA, M5G1X8; 3) Department of Molecular Biology and Genetics, Johns Hopkins University, Baltimore, MD, 21205.

In a screen to identify genes expressed preferentially in retina, we identified one, designated PHR1, that is of interest because it encodes 4 isoforms of 243, 208, 224 and 189 amino acids, all with pleckstrin homology (PH) domain at their N-terminus and a transmembrane domain at their C-terminus. Using in situ hybridization, immunohistochemistry, and immunoblot studies, we found isoforms 1 and 2 are integral membrane proteins in photoreceptor outer segments while isoforms 3 and 4 are abundant in brain. The PHR1 gene (11q13.5-14.1) utilizes two promoters and alternative splicing to produce 4 transcripts. In mouse, transcript 1 is comprised of 9 exons and originates from a 5' photoreceptor-specific promoter with at least three CRX elements, a cis-acting photoreceptor specific promoter element. Transcript 2 originates from the same promoter but lacks exon 7, which encodes 35 amino acids immediately C-terminal to the PH domain. Transcripts 3 and 4 originate from an internal promoter in intron 2 and either contain or lack exon 7, respectively. Human PHR1 has a similar genomic organization, except at the 5' end, where the sequence corresponding to murine exon 1, intron 1 and exon 2 forms the first exon. In human and mouse, transcripts driven by the internal promoter start in intron 2. To investigate PHR1 function, we performed in vitro binding assays with a series of PHR1 isoform 1 and 2 GST fusion proteins. We found no binding to inositol phosphates but specific binding to transducin bg subunits. Using truncated PHR1 fusion proteins, we located this activity to the PH domain. This binding activity together with abundant expression in the photoreceptor outer segment suggests that PHR1 may function as a modulator of the phototransduction. We have targeted murine PHR1 exon 3 and recently obtained heterozygotes. Study of these animals and their progeny should provide more information on PHR1 function.

Program Nr: 2171 from the 1999 ASHG Annual Meeting

Molecular regulation of human *single-minded* 2 (SIM2) gene. *A. Yamaki[1], J. Kudoh[2], S. Minoshima[2], N. Shimizu[2], Y. Shimizu[1]*. 1) Medical Genetics, Kyorin Univ Sch Health Sci, Tokyo, Japan; 2) Dept. Mol. Biol., Keio Univ. Sch. Med., Tokyo, Japan.

The single-minded 2 (SIM2) gene located on the human chromosome 21q22.2 is a human homolog of Drosophila sim gene which encodes a transcription factor essential for the development of midline cells in the central nervous system. We thus previously proposed the SIM2 is a good candidate gene for the pathogenesis of Down syndrome, particularly the mental retardation. Human SIM2 and mouse Sim 2 consist of 11 exons, spanning 50 kb and 40 kb in size, respectively. Mouse Sim 2 gene was expressed in the diencephalon of 8.5-day embryo and adult kidney, skeletal muscle, and hippocampus. Northern blot analysis revealed that human SIM2 mRNA was detected in fetal kidney, kidney, skeletal muscle, and prostate. As a step toward understanding the molecular mechanisms of SIM2 gene expression, we examined the transcription initiation site in the human SIM2 gene by 5'-RACE method using RNA isolated from the SIM2 gene-expressing glioblastoma cell line. The starting point of the transcription was found to be at 1.2-kb upstream of the initiation codon. To futher characterize the SIM2 promoter region, we made a series of deletion constructs by ligating with luciferase reporter gene. Transfection assay in the glioblastoma cells revealed that significant promoter activity located in the 43-bp sequence between nt -1307and nt -1349 upstream of the translation initiation site. Computer analysis in fact showed three cis-elements for c-myb, E-47 and E2F in the 43-bp sequence. We then made point mutations in each of these three cis-elements and found that a mutation in c-myb element was most affected the luciferase activity. The gel- shift assay using the 18-bp sequence containing c-myb element revealed at least two specific bands of oligonucleotide-protein complex. Thus, the c-myb transcription factor appears to be involved in the regulation of SIM2 gene expression in human glioblastoma cells.

Program Nr: 2172 from the 1999 ASHG Annual Meeting

Genomic structure of the human plasma prekallikrein gene, and association and mutation analysis in End-Stage Renal Disease. *H. Yu, B.I. Freedman, S.S. Rich, D.W. Bowden.* Biochem, Wake Forest Univ Sch Medicine, Winston-Salem, NC.

Kallikreins are serine proteases which catalyze the release of kinins and other vasoactive peptides involved in the regulation of blood pressure and renal perfusion. Previously, we have studied one tissue-specific and one plasma-specific human kallikrein gene in the End-Stage Renal Disease (ESRD). We found that polymorphisms for the human plasma prekallikrein gene (KLKB1, previously known as KLK3) on chromosome 4 were associated with ESRD in African Americans (Yu et al., Hypertension 1998;31:906-911). We have extended the study of the role of KLKB1 in ESRD. First, we determined the exon-intron boundaries of KLKB1. Second, based on this information of gene structure, we designed primers to amplify KLKB1 exons. These primers were used to screen for mutations in the ESRD patients. The exon-intron boundaries were determined using the Polymerase Chain Reaction (PCR) with primers designed from KLKB1 cDNA. Sequence of the 5' region of KLKB1 was extended by shotgun-cloning. Mutations were detected using the Single Strand Conformation Polymorphism (SSCP) method. All exon-intron boundaries have been determined. Like the rat gene, the human plasma kallikrein gene contained 15 exons and 14 introns. The longest intron, F, was almost 12 Kb long. The total length of the gene was approximately 30 Kb. Two new "CA/GT" repeat markers were identified within the gene, and designated as KLK3f and KLK3g. They had heterozygosities of 0.65 and 0.84, respectively. We have detected 8 allelic variants in 7 exons, with a major one at position 521 of KLKB1 cDNA. This mutation (A521G) replaced an asparagine with a serine at position 124 in the human plasma prekallikrein peptide. Results using the Relative Predispositional Effect (RPE) technique indicated that the frequencies of alleles 4, 8 of KLK3f and allele 8 of KLK3g were significantly different between controls and ESRD cases. They accounted for 0.226, 0.096 and 0.313, respectively, in the probands of 166 ESRD families as compared to 0.172, 0.066 and 0.244 in 139 race-matched controls (allele P and total $P < 0.05$ for all three alleles). Therefore, KLKB1 might contribute to the risk of developing ESRD in African Americans.

Program Nr: 2173 from the 1999 ASHG Annual Meeting

Patient willingness to contact at risk relatives for genetic testing studies: experience with hemophilia A. *T. Jennings-Grant, N. Callanan, C. Lakon, I. Lubin, J.R. Sorenson, T. Spinney, G. White.* University of North Carolina at Chapel Hill, Chapel Hill, NC.

As part of a NHGRI funded study 127 hemophilia A patients (or parents if a minor) with hemophilia A were asked to participate in a study in which genetic counseling and carrier testing would be offered at no charge to their at risk female relatives. For patients, participation was limited to providing family history and giving consent to have their medical chart reviewed, submitting a blood sample for DNA mutation analysis and completing a single intake questionnaire. Twenty-nine of the 127 did not meet study inclusion criteria. Of the remaining 98 patients 66 (67%) agreed to participate. Preliminary analysis of the data suggests that participation is not influenced by race, education or religion. However, the participation rate was 89% for patients ³ 19 and 58% for those £ 18 years of age. 89% of patients with severe and 63% of those with mild or moderate hemophilia A participated. Overall, 98% reported that someone had previously talked with them about the inheritance of hemophilia, and 84% had been informed that female relatives might be carriers. Among those who had not received prior information about the carrier risks for relatives 93% participated. 30% think their relatives have a high or extremely high risk for having a child with hemophilia, and 60% have previously discussed carrier testing with their relatives. Overall, 84% thought they would be able to contact relatives about the study and 88% did not think that our asking them to do so was an invasion of their or their relatives' privacy. Of the 71% of patients who felt that they had an obligation to inform relatives about the study 78% participated whereas 59% of those who did not feel this obligation participated. The implications of these data for recruiting at risk relatives into gene testing studies will be discussed.

Program Nr: 2174 from the 1999 ASHG Annual Meeting

Dilemma to chronic ventilation in Werdnig-Hoffmann disease: study of 16 patients. *C.E. Kim, P. Lecussan, E. Casella, L. Albano, G. Oselka.* Pediatrics, Instito da Crianca, Sao Paulo, Brazil.

Werdnig-Hoffmann disease, the most common lethal autosomal recessive disorder and its frequency is 1 per 10 000 births. Affected patients usually die before 2 years of age, due to the respiratory involvement. In Brazil, due to cultural,religious and legal problems, almost all patients are submitted to chronic ventilation and have their lives actively prolonged for unlimitated time.Therefore,iniciation of ventilator use in this condition is raising an important ethical issue and high medical costs. We studied 16 patients, all under permanent mechanical ventilation, started from 2 to 19 months (mean age=6,6 mo). Ten patients are maintained in intensive care units of hospitals and 6 patients are at home care. The duration of ventilation varied from 7 months to 13 years. The average time of ventilation was 6 years in patients at home and 2 years and 9 months in patients at hospital.Two among the patients in hospital died after 4 and 21 months of ventilation. The current ages of alive patients range from 1 year and 5 months to 14 years. Tracheostomy and gastrostomy were performed in all patients. interestingly, the parents of all patients prefere their child to be submitted to ventilation, even thoughsuffering emotional stress and poor quality of the life style without any pespectives of improvement.

Program Nr: 2175 from the 1999 ASHG Annual Meeting

The Development of a Web-Based Family Health History Collection Tool. *W. Knaus[1], W. Cohn[1], J. Barrett[1], M. Kinzie[2], S. Pelletier[1], M. Julian[2], J. Einbinder[1].* 1) Health Evaluation Sciences, University of Virginia, Charlottesville, VA; 2) Department of Educational Studies.

The human genome project is providing a wealth of information about the genetic basis of common diseases. This information has implications for clinical practice however; it will only be useful to physicians and patients when coupled with family history information. The effective use of family history information in the primary care setting will depend on to factors. The first is the ability of the patient to collect complete family health history information. The second is the ability of the primary care physician to interpret this information to provide specific recommendations. The Family Health History Collection Tool is designed to improve the collection and interpretation of family health history information. This web tool will provide an easy and efficient way for patients to collect, maintain and update their family health history. Additionally, this tool will apply interpretive algorithms to stratify patients' risk for the most common diseases. Algorithm development involved specialists in oncology, endocrinology, neurology, and cardiology. A geneticist and genetic counselor participated in the development phase of both the algorithms and the system design. Primary issues related to the development of the system included; the necessity of involving professionals from many fields due to the complexity of the project; conducting a thorough needs assessment of stakeholders including all potential users; developing a strategy to balance the needs and professional interests of each stakeholder and designing a process for algorithm development in each specialty area.

Program Nr: 2176 from the 1999 ASHG Annual Meeting

How Effective is Continuing Education in Genetics? The HuGEM II Experience. *C. Kozma[1], E.V. Lapham[1], J.O. Weiss[2], J.L. Benkendorf[1], M.A. Wilson[2].* 1) Georgetown Univ Medical Center, Washington, DC; 2) Alliance of Genetic Support Groups, Washington, DC.

As part of the Human Genome Education Model Project (HuGEM) II, (a project of Georgetown University Medical Center and the Alliance of Genetic Support Groups), 18 three-hour continuing education workshops were held in 9 states in 1998-1999 for over 600 health professional practitioners. Attendees were primarily dietitians, occupational and physical therapists, psychologists, speech-language pathologists and audiologists, and social workers. At the beginning of each workshop, participants were asked to complete four questions about their prior knowledge of genetics and how important they considered genetic issues to be to society and to their professions. Responses from 423 attendees showed that 40% knew a lot about genetics prior to the workshop and 60% said they knew almost nothing. Those who knew a lot were most likely to be in their 50s (49%) and least likely to be in their 20s (32%). More women (87%) than men attended the workshops and more women (41%) than men (36%) knew a lot. Those who knew a lot considered genetic issues to be more important to society (81% versus 61%) and to their profession (69% versus. 55%). However, by the end of the workshop the gap was narrowed so that 86% of those who knew a lot and 83% of those who knew almost nothing considered genetic issues to be very important to society. And 79% and 71% respectively considered genetics to be very important to their professions. A serendipitous finding was related to individuals who were least satisfied. While 90% were very satisfied with the speakers, topics and information, the 10% who were least satisfied (1-3 on a 5-point scale) went from 44% who considered genetics very important to society before the workshop to 71% after. The change was 35% to 48% who considered genetics very important to their professions. These findings counter the prevailing wisdom that satisfaction equals learning. Additional studies and measures are needed to consider this paradox.

Program Nr: 2177 from the 1999 ASHG Annual Meeting

Contacting at risk relatives for a genetic counseling and testing study: experience with hemophilia A patients.
C.M. Lakon, N. Callanan, T. Jennings-Grant, I. Lubin, J.R. Sorenson, G. White. University of North Carolina at Chapel Hill, Chapel Hill, NC.

One means of recruiting high-risk populations into genetic testing research is to ask patients to inform at-risk relatives about the research. As part of an ongoing hemophilia A study in which genetic counseling and carrier testing is offered at no charge to at risk female relatives, we have contacted 127 hemophilia A patients (or their parents if a minor). Of the 98 patients who met eligibility requirements, 66 (67%) agreed to participate. Mutation analysis has been completed for 59/66 participants and a disease causing or associated mutation has been identified in 50(84.7%). We have been unable to contact 2 patients (11 at risk relatives) with their results. The remaining 48 patients have 341 (average 7.1 /patient) at risk relatives. Patients were asked to inform relatives about the study and obtain their consent to be contacted by the study team. To facilitate this process, patients were provided with a list of at risk relatives, a fact sheet about the study to share with relatives and were reimbursed for the cost of contacting relatives. Thus far 63% (32/48) have supplied us with consent and information to contact 163 (47.8% of total) at risk relatives. There were 77 (22.6%) relatives who were informed about the study but did not wish to participate. Patients or their designees were not willing or not able to contact 37(10.8%) relatives. 19 (5.6%) relatives were contacted by the patient but did not respond within the time limit to be included in the study. There were 45(13.2%) relatives for whom the patient did not provide contact information within the time limit for inclusion in the study. 16/48 patients (33%) have not supplied contact information for any relatives. Of these, 8 report that none of their relatives wish to participate, 1 has refused to contact relatives, and 7 have missed deadlines for providing contact information to the study team. The implications of these data for recruiting at risk relatives into gene testing studies will be discussed.

Program Nr: 2178 from the 1999 ASHG Annual Meeting

Do laws restricting health insurers' use of genetic information reduce the fear of discrimination and increase genetic testing? *J.S. Lawlor, S.S. Rich, M.A. Hall.* Public Health Sciences, WFU School of Medicine, Winston-Salem, NC.

Methods: Qualitative, comparative, case-study analysis in seven states, with varying laws restricting health insurers' use of genetic information. Expert, semi-structured interviews were conducted in 1998 with 29 genetic counselors or medical geneticists and five patient advocates. Informed consent forms and patient information brochures used by medical geneticists were content analyzed. Also, a review was conducted of published articles and unpublished studies based on surveys done at genetic clinics, concerning factors that influence testing decisions and perceptions of the threat of genetic discrimination. **Findings**: (1) Patients' fear of genetic discrimination greatly exceed the reality, at least for health insurance. (2) Whether these fears deter genetic testing depends on the costs and benefits of testing. The greatest difference is to those who do not want to submit the costs of testing for reimbursement and who cannot afford to pay for testing out of pocket. There appears to be little deterrence for tests that are more easily affordable or when the need for the information is much greater. (3) Fear of discrimination plays virtually no role in testing decisions in pediatric or prenatal situations. Fear of discrimination is significant only for adult-onset conditions, and for adults there is less concern when there are existing symptoms or when indications of the condition are already documented in the medical record. (4) There is widespread awareness of these laws among genetic counselors, but counselors' knowledge of the basic provisions is not accurate, and they frequently underestimate how protective these laws are, as written. **Conclusion**: These laws have not greatly reduced the fear of discrimination in part because people in the medical genetics community are not confident these laws will actually prevent discrimination until there are test cases of actual enforcement. Ironically, there may be so little actual discrimination that it is not possible to initiate good test cases. If the fear of discrimination were lessened, this would likely have a strong impact on patients' decisions to undergo testing only in a limited range of conditions.

Program Nr: 2179 from the 1999 ASHG Annual Meeting

Establishment of a dedicated cancer genetics program in a tertiary pediatric centre. *D. Malkin[1], K. Smyth[1], C. Shuman[2], N. Quercia[2], K. Australie[3], R. Weksberg[2].* 1) Hematology/Oncology and; 2) Clinical Genetics, The Hospital for Sick Children, Toronto, Ontario, Canada; 3) Clinical Genetics, Montreal General Hospital, Montreal, Quebec, Canada.

The Hospital for Sick Children is a tertiary care medical center that serves a population of 8 million. The Division of Oncology admits and treats ~325 newly diagnosed cancer patients annually. Although the cause of childhood cancer is for the most part not known, previous reports have suggested that 5-10% may have a genetic etiology. In a recently completed study of the impact of predictive genetic testing on parents of children with cancer,we observed that parents' perceptions of genetic risk for cancer were innacurate. Furthermore, pediatric oncologists' understanding of a child's right to know the results of genetic screening, of the potential value of genetic information, and of the accuracy of the family cancer history were all incomplete. Based in part on this data, we developed a unique Cancer Genetics Program and Clinic within the Division of Oncology. The primary objective of the Program is to establish an infrastructure to facilitate accurate genetic counseling of families and development of improved education of pediatric oncology health care professionals. A secondary objective is to foster fundamental and clinical genetic interdisciplinary research studies based on a comprehensive database. All newly diagnosed patients and their parents are referred to the clinic for complete pedigree analysis and counseling. A genetic component to the occurrence of cancer is suggested by the family histories taken to date of at least 25% of probands. Furthermore, previously unrecognized clinical characteristics suggestive of a genetic syndrome have also been observed. The clinic has enhanced the presence of clinical genetics in the pediatric cancer setting. We conclude that development of a cancer genetics program aligned with both Oncology and Clinical Genetics in a large tertiary care pediatric centre is feasible. It fascilitates the education of parents and families to the issues of genetic risk and testing, and enhances opportunities for research into the genetic basis of childhood cancer.

Program Nr: 2180 from the 1999 ASHG Annual Meeting

Feeling to blame: do genetic doctors and nurses discuss it during genetic counselling and should they? *C. Metcalf[1], J. Hewison[1], R.F. Mueller[2], A. Walker[3], H. Cuckle[4].* 1) Sch of Psychology, Univ of Leeds, Leeds, England; 2) Dept of Clinical Genetics, St James's Hospital, Leeds, England; 3) Health Services Research Unit, Univ of Aberdeen, Aberdeen, Scotland; 4) Dept of Reproductive Epidemiology, Univ of Leeds, Leeds, England.

Statement of Purpose Unresolved and inappropriate blame for health conditions can have long term psychological consequences. In the case of inherited disorders, the potential for parents and other family members to blame themselves, or one another, is high. A potentially important function of genetic counselling in these circumstances is to address and allay this propensity for blame.

Methods Used This paper reports findings from a UK national study involving ten regional genetics units and 107 cases of genetic counselling: 38 for X-linked disorders (Duchenne muscular dystrophy and Fragile X syndrome), 38 for cystic fibrosis and 31 for chromosomal rearrangements. Post-session questionnaires were obtained from 131 individuals and 100 genetic counselling sessions were recorded on audiotape, transcribed and analysed. Psychological status was measured using the Hospital Anxiety and Depression (HAD) scale.

Summary of Results There was evidence of self-blame in almost one third of cases (31/107). Self-blame was reported in 26 questionnaires but from the corresponding session transcript, half (13) these individuals neither mentioned self-blame, nor were invited to discuss it. Psychological well-being, assessed by HAD anxiety scores, was significantly worse in self-blamers (self-blame mean = 9.2, no self-blame mean = 4.5, $t = 5.35$, $p<0.001$).

Conclusions Self-blame is a common experience among consultands for genetic counselling and is associated with high levels of anxiety. In a number of cases, there is no mention of self-blame by the individual nor encouragement from the doctor or nurse leading the session. This might indicate that the professionals taking the session are not identifying such a psychologically important issue. Left undetected, self-blame and its adverse psychological impact could have lasting implications for mental health.

Program Nr: 2181 from the 1999 ASHG Annual Meeting

Using Multimedia to Enhance Teaching of Contemporary Medical Genetics. *S.A. Metcalfe[1], R. Williamson[1], A. Bonollo[2]*. 1) Murdoch Institute,Victoria, Australia and Dept Paediatrics, Faculty of Medicine, University of Melbourne, Australia; 2) Biomedical Multimedia Unit, Faculty of Medicine, University of Melbourne, Australia.

Rapid advances in human genetics and medical applications of genetic technologies provide new challenges for medical and allied health educators. Lecture-based teaching may not provide sufficient time or depth for students to grasp complex concepts and students are often reluctant to engage in interactive dialogue on social/ethical issues of genetics. We are developing a self-directed learning multimedia package that integrates basic concepts of genetics within a clinical framework. Specific single-gene and chromosomal disorders are used to highlight molecular basis, principles of genetic testing, and counselling issues, which are incorporated into 3 modules: i) Clinical Diagnosis, ii) Laboratory Diagnostics, iii) Counselling and Ethical Issues. The CD-ROM features interactivity, animations and text delivered in a number of ways, including 'rollovers', feedback, and glossary. Various media enhance visual diversity: photos of affected persons, images of molecules, DNA test results, and videos of counselling methods/sessions. Interactive problem-solving exercises are offered, often using the 'drag-and-drop' approach, such as building pedigrees from family histories, karyotyping chromosomes, analysing/understanding molecular basis of DNA tests and multiple choice questions for self-testing. Incorrect solutions are possible at the interactive steps with on-screen feedback. In the counselling module, students can view video clips of scripted genetic counselling sessions with 'time-outs' highlighting key issues. The program is still in its development stage but has been used in a computer lab setting for 1st year medical students. Evaluation is by content and evaluation experts, and students who have been educated in an 'older style' curriculum. This multimedia program allows students to perform problem-solving exercises, explore counselling/ethical issues, and study medical genetics at their own pace, thereby providing a flexible learning environment to enhance self-study and lifelong learning skills.

Program Nr: 2182 from the 1999 ASHG Annual Meeting

ATTITUDE TOWARDS PROPHYLACTIC SURGERY AND EFFECTS OF GENETIC COUNSELLING IN FAMILIES WITH BRCA MUTATIONS. *R. Moeslinger[1], T. Wagner[1], G. Langbauer[1], Austrian HBOC Group[1], A. Auterith[3], A. Friedmann[2], C. Zielinski[4], M. Seifert[1], P. Oefner[5]*. 1) Dept Gyn OB, Div Senology, Univ Vienna, Vienna, Austria/Europe; 2) Dept Psychiatry, Univ Vienna, Vienna, Austria; 3) Dept of Medical Statistics, Univ Vienna,; 4) Dept Medicine I, Div Oncology, Univ Vienna; 5) Dept Biochemistry, Univ Stanford, Stanford.

The intent of this study was to evaluate the effect that awareness of being a BRCA1 or BRCA2 mutation carrier has on the attitude towards prophylactic surgery, as well as on developing depression symptoms, body image, and the ability to cope with carrier status. Thirty-five families were selected on the basis of previously detected BRCA1 or 2 mutations and 90 family members were given the appropriate questionnaires. Prophylactic mastectomy (PM) was considered by 21% of the Austrian mutation carriers (29% affected and 8% non-affected carriers). The majority of affected and non-affected carriers expected PM to impair the quality of their life. Fifty percent would undergo prophylactic oophorectomy (53% affected and 46% non-affected carriers). The self-rating depression scale indicated that following mutation result disclosure the depression scores of carriers decreased (40 baseline vs. 38 after result disclosure, p=0.3), whereas, for non-carriers, scores increased (36 baseline vs. 40 after result disclosure, p=0.05). We conclude, that information about carrier status is not associated with increased depression symptoms in mutation carriers. In non-carriers depression scores increased slightly, probably reflecting survivor guilt. The option of having PM was associated with a negative impact on the quality of life and was declined by the majority of Austrian mutation carriers.

Program Nr: 2183 from the 1999 ASHG Annual Meeting

Genetics in the Nursing Literature: A Source of New Knowledge for Practice, Education, and Research. *R.L. Monsen[1], G. Anderson[2]*. 1) University of Arkansas for Medical Sciences, College of Nursing Little Rock, AR 72205; 2) Stanford Center for Biomedical Ethics Palo Alto, CA 94304.

Nurses are regarded as an important resource in meeting the disease-specific requests for information and assistance as gene-based diagnosis and therapies become routine health care practice. Nurses are more numerous in community health settings than are genetic specialists and have the capacity to acquire the basic concepts needed for recognizing the need for referrals and accessing appropriate sources of genetic information for use by consumers. Moreover, as the need for individual counseling and follow-up accompanying genetic testing for frequently occurring conditions, nurses can be called upon to provide these time-consuming services in collaboration with certified genetic specialists. The nursing literature is one of the most important sources of new knowledge for nursing practice, education, and research. Therefore, the purpose of this ELSI funded study was to conduct the first integrative review of genetics in the nursing literature from its inception in 1962 to the end of the 20th century. Over 500 citations published were ascertained from multiple online bibliographic databases. In this paper, the authors provide statistics about the genetic topics of most concern to these nurse and non-nurse authors, a description of the type and diversity of authorship, a description and a conceptual map of the types of clinical practice models now used by nurses who use genetic information in their practice, and interventions recommended by these authors for the entire discipline of nursing for people who have health care concerns related to genetics. The areas of strength in this nursing literature include cancer genetics, prenatal diagnosis and reproductive technologies, and single gene disorders frequently encountered in children and adolescents. Recommendations for preparing the nation's nurses to assume roles in case-finding, support counseling, and follow-up of genetic services are among the most important next steps in the era of geneticized health care.

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Program Nr: 2184 from the 1999 ASHG Annual Meeting

Psychosocial effects of type 1 diabetes genetic risk information: a pilot study. *L. Nicol-Smith[1], O. Søvik[2], G. Joner[1], K. Tambs[1], K.S. Rønningen[1]*. 1) Dept. of Population Health Sciences, National Institute of Public Health, Oslo, Norway; 2) Dept. of Pediatrics, Haukeland University Hospital, Bergen, Norway.

In a nationwide project in Norway, approx. 2,200 first-degree relatives of children with type 1 diabetes were HLA-DQ typed between 1994 and 1997. 20 pediatric departments were involved in collecting blood samples and relaying results. Risk information has been relayed, in part, back to the participants. The long-term impact of this project on both participants and physicians is being examined. A questionnaire for the parents who have undergone testing has been developed which evaluates perceived risk, affective response to test result and subjective health. Structured interviews will be undertaken with the pediatricians. Thus far data from one county in Norway has been collected. Of 127 pilot questionnaires that have been sent out 52 (41%) have been returned. Preliminary analysis reveals that 13 (25%) parents report thinking about their test result at least once a month, and 6 (11%) are slightly troubled by these thoughts. None regret being tested, but 2 would not recommend testing to a friend. Understanding of test results appears to be good. No systematic differences between those with perceived increased risk (n=19) and those with normal risk is indicated using standardized measures of anxiety, depression and subjective health. The pilot study has so far shown: 1) a few of the participating parents receiving information about their disposition to type 1 diabetes are "troubled" about their result two years afterwards; 2) methods which have been developed to evaluate psychosocial effects of genetic risk information in a specialist clinical genetic setting may be inadequate for monitoring the population effects of widespread genetic dispositional testing for common disorders; 3) the participating pediatricians have shown varying degrees of unpreparedness to give information regarding genetic susceptibility.

Program Nr: 2185 from the 1999 ASHG Annual Meeting

Clinical Genetics in a Family Practice Residency. *E.J. Palmer.* Family Med, Allegheny Fam Phys, Altoona Hosp, Altoona, PA.

Although referral to a genetics center may be indicated for complex cases, genetic health care, including counseling, for most common problems, should be provided by primary care physicians. Family Practice physicians, faculty, and residents may, however, lack a basic knowledge of clinical genetics and genetic testing, which could result in inappropriate responses to clinical situations in which a significant genetic component is evident. The following study was undertaken to assess the problem. First, a 10 question survey about the Marfan Syndrome was distributed to 72 community family physician attendees at an annual State Medical Society meeting. The survey acknowledged a general lack of formal clinical genetics training in medical school and residency, and only 55% of the questions about Marfan's Syndrome were answered correctly. Second, a poll of 74 Family Practice residency programs demonstrated no specific clinical genetics components in the curriculum. Third, the 50 question quiz from the AAFP Clinical Genetics monograph was administered to 6 faculty and 20 residents at a Family Practice residency program and yielded an average score of 53% correct for faculty and 42% correct for residents. Finally, a retrospective review of 176 obstetrical patient's charts was performed at a Family Practice residency. Of the 36 charts with recorded genetically inherited disorders listed in the family history, an appropriate clinical response was documented for only 33% of the cases. A structured, 3 year, curriculum in clinical genetics has been designed for a residency program. Assessment of the effectiveness of the program includes tests of knowledge base, demonstration of counseling skills, use of resources, audits of charts, and surveys of graduates. Since the primary care physician is usually the first person a patient seeks with medical questions, it is necessary for Family Physicians to assimilate and integrate the information and technology arising from the Human Genome Initiative.

Program Nr: 2186 from the 1999 ASHG Annual Meeting

Genetic education needs for primary care providers: A pre-intervention survey. *B.A. Pletcher, C.L. Koval, G. Gilbert, G. Rodriguez.* Center for Human & Molecular Genetics, University of Medicine and Dentistry- New Jersey Medical School, Newark, NJ.

As part of a federally funded genetic education project for primary care practitioners,78 participants completed a pre-intervention survey to assess their genetic education and service needs. Providers included 27(35%) pediatricians, 18(23%) OB nurse practitioners or midwives, 14(18%) family practitioners, 8(10%) internists, 5(6%) obstetricians, 5(6%) pediatric nurses and 1(1%) internal medicine nurse. Practice trends suggested that obstetricians by far had the greatest numbers of patients requiring testing or genetic referral. In contrast, obstetric nurses had many fewer patients requiring testing or genetic referral. Pediatric providers had intermediate levels of genetic testing and consultation needs whereas family practitioners were more likely to do genetic testing themselves and infrequently referred for genetic consultation. Internal medicine providers tested and referred the fewest patients. Twelve(15%) of respondents did not know where to refer patients for genetic services and 40(51%) did not know what genetic tests are available to patients. In the preceding year 15(19%) had procedural difficulties getting genetic testing done for their patients and 19(24%) had financial problems in this regard. Although 48(61%) stated they had a genetic course as part of their professional school curriculum, only 3(4%) received any CME credits in the past year related to genetic topics. Thirty-two(41%) of respondents have no internet access in their practice settings, 44(56%) do not use the worldwide web regularly and 51(65%) do not even use email regularly to communicate with other clinicians. In summary, in the course of developing a primary care provider educational program it became clear that providers in our region need a) to learn about genetic testing availablity b) to have more genetic offerings as part of CME programs and c) to have information communicated in ways other than through the internet or via email. Genetic testing and referral patterns varied significantly between specialties and these services are used the least by adult medicine specialists.

Program Nr: 2187 from the 1999 ASHG Annual Meeting

Sibling knowledge and attitudes toward carrier testing for X-linked severe combined immunodeficiency. *J.M. Puck[1], J.H. Fanos[2], J. Davis[1]*. 1) NHGRI/NIH, Bethesda, MD; 2) California Pacific Medical Center Research Institute, San Francisco, CA.

X-linked severe combined immunodeficiency (XSCID) is the most common genetic form of SCID, a rare (1/50,000 births) disease with profoundly impaired cellular and humoral immunity. Popularized as the disease of David the Bubble Boy, isolated from birth in a germ-free environment, SCID was previously fatal, but now is usually successfully treated by bone marrow transplantation. Mapping of XSCID in 1985, and identification of the disease gene, IL2RG, in 1993, have made possible patient and carrier diagnosis. We assessed understanding of the genetics of XSCID and attitudes toward carrier testing in siblings recruited from families in which a proband had previously enrolled in our linkage or mutation detection protocols and been proven to have XSCID. 37 female and 3 male adult siblings completed a questionnaire and semi-structured interview. Overall knowledge of the genetics of XSCID was excellent, with 14 of 16 questions answered correctly by >75% of subjects. Questions frequently missed: only 49% knew all daughters of an affected boy would be carriers, and 53% were unaware that spontaneous mutation for XSCID was possible. Of interest, siblings from kindreds with a documented spontaneous mutation were less likely to answer this question correctly than those in multi-generational XSCID kindreds (p=0.01). 93% of our sibling group believed daughters should be tested for XSCID carrier status. 89% would prefer to have their own daughter tested prior to reaching 18 years (M=9, Median=12); 34% would test at birth. 89% would disclose carrier results to their daughter before her 18th birthday (preferred disclosure age M=12 years, Median=12); 51% would disclose prior to adolescence. Preference for carrier testing before adulthood reflected the subjects' desire to have parents impart knowledge at what they felt was an optimal time to minimize childrens misconceptions. We recommend that genetic counseling for XSCID include children in age-appropriate discussions and help parents weigh the benefits of early testing and disclosure vs. the potential harm of loss of child autonomy.

Program Nr: 2188 from the 1999 ASHG Annual Meeting

A Critical Analysis of the Research Performed on Psychosocial Aspects of DNA Testing for BRCA1/2. *D. Punales-Morejon, G. Havens.* Division of Medical Genetics, Beth Israel Medical Center, New York, NY.

Mutations in the BRCA1/2 genes increase the risk of developing certain cancers. Several factors that predict whether a woman will elect genetic testing for BRCA1/2 have been studied. Investigators have identified the following variables as impacting a woman's decision-making process: level of education, having health insurance, number of first degree relatives affected by breast and/or ovarian cancer, perceived versus objective risk of being a mutation carrier, perceived susceptibility of ovarian cancer and/or recurrent breast cancer, level of cancer genetics knowledge, perceived benefits versus limitations of genetic testing, and psychological distress.

However, these studies suffer from limitations which impact on their generalizability. For example, most studies have asked subjects only to consider hypothetically whether they would pursue testing. Yet the literature clearly demonstrates a significant difference between hypothetical consent and actual test use. Other limitations in the prior studies include subjects that were overly diverse regarding cancer risk and knowledge, and various predictive factors were not tested simultaneously.

While it is evident that there is limited information available about the variables that predict utilization of DNA testing, even less is understood about the mechanisms involved in the decisional process. Understanding decisional variables has important clinical implications. Some predictor variables may have greater relative strength than others and as such have different impact potential in decision making. From this analysis of the current research on psychosocial issues in genetic testing for breast and ovarian cancer, a set of recommendations is derived for the purpose of advancing the understanding of the psychological and social factors involved in the pursuit of DNA testing. This understanding may allow genetic counselors and geneticists to better help women and their families make critical decisions in the growing arena of cancer genetics and susceptibility testing.

Program Nr: 2189 from the 1999 ASHG Annual Meeting

Implementation of an inherited breast/ovarian cancer susceptibility clinical practice guideline: Who receives counseling, and who is interested in genetic testing? *J. Reiss[1], J. Bergoffen[1], D. Broome[1], E. Harris[1], A. Mims[1], U. Ochs[2], S. Rowell[1]*. 1) Kaiser Permanente (KP) Health Plans—California, Georgia, and Northwest; 2) Group Health Cooperative (GHC), Seattle WA.

KP and GHC provide comprehensive health care for over 9 million persons. A registry of individuals counseled for genetic susceptibility was developed as part of implementing KP's and GHC's clinical practice guidelines for counseling for inherited breast/ovarian cancer susceptibility. Its purpose is to provide a resource for guideline evaluation and research studies. Clinicians (primarily genetic counselors and clinical geneticists) complete a standard form for each person counseled, and submit it to a central database. Regular contributions started between December 1997 and January 1999, depending on the area. As of June 1999, the registry contained forms for 208 persons who received individual counseling.

Referrals most often came from obstetrics/gynecology (35%), internal medicine (25%), and oncology (15%); 6% self-referred. Nearly all counselees were women (99%); median age was 48 years. Most were white (89%); 20% were Ashkenazi Jewish. 31% had breast or ovarian cancer, and 83% had at least one first-degree relative with breast or ovarian cancer. 41% of those counseled met the guidelines; in addition, 20% were judged to be high-risk for carrying a deleterious BRCA1 or BRCA2 mutation or another inherited cancer susceptibility gene. 49% were "definitely interested" in genetic testing, including 17% judged not to be high-risk. 70% of those with breast/ovarian cancer, and 38% of those with only a family history of breast/ovarian cancer, were definitely interested in testing. Our findings suggest that most individuals are being referred appropriately, but a substantial proportion of those counseled (39%) may not be. Future work needs to assess reasons for these referrals, and methods to minimize low-risk referrals without adversely affecting quality of care (e.g., more effective methods of clinician and/or patient education).

Supported by Permanente medical group, Garfield Memorial Fund, and KP Center for Health Research.

Program Nr: 2190 from the 1999 ASHG Annual Meeting

Parental Attitudes Towards a Diagnosis in Children with Unidentified Multiple Congenital Anomaly Syndromes.

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Approximately 1% of newborns are affected with multiple birth defects. In over half of these cases it is impossible to identify a specific syndrome to explain the pattern of anomalies. Genetics clinics provide care for many children with unexplained multiple congenital anomaly syndromes, but little is known about the ways in which the lack of a diagnosis affects parental coping and adjustment. We interviewed the parents of 16 children born with an unidentified multiple congenital syndrome in order to learn more about their attitudes towards diagnositic information. The parents in our sample had all been aware of their child's birth defects for between 2 and 23 years, and they had all sought multiple evaluations in an attempt to find a diagnosis that could explain their child's condition. Since our sample was selected from a pool of parents who had already demonstrated an interest in diagnositic information it was not surprising that most of them told us that they are still interested in identifying their child's syndrome. Typically, they told us that they had been especially anxious to obtain a diagnosis when the child was younger, but their interest has diminished to varying degrees with the passage of time.

Using qualitative methodology, we identified six general areas where the parents feel that a diagnosis would have significant impact: Labeling, Causes, Prognosis, Treatment, Acceptance, and Social Support. Some of the most significant issues revolved around obtaining special education services, knowing what to expect in their child's future, watching out for new problems, fears about the child's life-expectancy, concern about recurrence risks, a desire to make sure that the child was receiving appropriate treatment, and feelings of isolation. Parents did not always feel that a diagnosis would help them deal with these issues, and in some cases they felt that a diagnosis could be harmful.

Program Nr: 2191 from the 1999 ASHG Annual Meeting

Experience of Support Persons Participating in Presymptomatic Genetic Testing Protocols for Huntington Disease. *D.L. Schutte[1], J.K. Williams[1], P.A. Holkup[1], C. Evers[2,3], A. Muilenburg[3]*. 1) College of Nursing, The University of Iowa, Iowa City, IA; 2) Iowa Regional Genetics Consultation Service, Iowa City, IA; 3) Department of Pediatrics, The University of Iowa, Iowa City, IA.

The identification of genes associated with adult-onset conditions, such as Huntington disease (HD), enables presymptomatic genetic testing for at-risk healthy individuals. Genetic testing protocols for HD, including pre- and post-testing genetic counseling visits, were developed to integrate this new technology into clinical practice. Most presymptomatic genetic testing protocols for HD recommend that a support person accompany the individual seeking testing throughout the genetic counseling and testing process. While the experiences of persons undergoing presymptomatic genetic testing are described in the literature, little is known about the experiences of the support person. The purpose of this cross-sectional descriptive pilot study is to describe the experiences of support persons participating in presymptomatic HD genetic testing protocols. Semi-structured interviews and quantitative measures of emotional distress were analyzed for 18 subjects. Content analysis of transcribed interviews revealed that support persons cited the importance of being there for the person undergoing testing and reported a variety of strategies to provide support. Despite recognizing the importance of the role, most support persons had not formally prepared for the experience and suggested the need for peer counseling and anticipatory guidance as a means of doing so. Several services were endorsed as potentially helpful either during or following the testing process, including spiritual support, additional counseling, support groups, and financial counseling. Psychological measures indicate worry and distress related to the genetic testing event similar to at-risk persons undergoing presymptomatic genetic testing. Results from this pilot study suggest potential strategies for refinement of existing presymptomatic genetic testing protocols in order to enhance the experience for the persons seeking genetic testing as well as their support persons.

Program Nr: 2192 from the 1999 ASHG Annual Meeting

Family history of breast cancer as a risk factor for breast cancer in Arab women in Israel. *T. Shohat[1], M. Aghassi-Ippen[1], M.S. Green[1], M. Shohat[2]*. 1) ICDC, Israel Center for Disease , Tel-Hashomer, Israel; 2) Genetic Institute,Rabin medical center, Petach tikva , Israel.

While the incidence rate of breast cancer in Jewish women in Israel was 82.3 per 100,000 in 1993, the incidence in Arab women living in Israel was 22.4. One of the possible explanations for this difference is that previously described risk factors for breast cancer do not play a major role in the Arab population. Family history of breast cancer is one of the major risk factors known for this disease. 10-15% of women with breast cancer have a mother and/or a sister with breast cancer. The aim of the study was to investigate the association between family history and breast cancer in Arab women in Israel. 72 women diagnosed with breast cancer aged 30-70 and 140 controls without breast cancer or any other cancer in the same age group were studied. The cases were identified through oncology wards and the controls were recruited from Kupat-Holim and hospital clinics. All study participants were interviewed by a questionnaire which included questions regarding family history of cancers and details on other known risk factors. Eight women with breast cancer and 5 women without breast cancer had a first degree relative with breast cancer. The odds ratio for breast cancer for a woman with a first degree relative with breast cancer was 3.4, (P = 0.04). No association was found between other variables studied (age of menarche, number of births etc.) and breast cancer. The risk for breast cancer for first degree family members of Arab patients with breast cancer is in the same magnitude of the risk that was found for family members in the Jewish population around the world.

Program Nr: 2193 from the 1999 ASHG Annual Meeting

Knowledge and attitudes of Hispanic women and their health care providers about breast cancer risk factors and screening. *M.N. Strecker[1], A.J. Tucker[1], M.L. Bondy[2], D.A. Johnston[3], H. Northrup[1]*. 1) Department of Pediatrics, The University of Texas Houston Medical School, Houston, TX; 2) Department of Epidemiology, The University of Texas M.D. Anderson Cancer Center, Houston, TX; 3) Department of Biomathematics, The University of Texas M.D. Anderson Cancer Center, Houston, TX.

Medically underserved women, specifically those individuals who lack medical insurance, are of lower socioeconomic status, and have less education, frequently face significant barriers to obtaining appropriate breast cancer screening. In order to address the needs of an underserved population, we designed and implemented educational programs for the lay public and for health care providers about breast cancer risk factors, screening guidelines, and implications of hereditary predisposition to breast cancer.

Our specific aims were to assess the knowledge and attitudes of medically underserved Hispanic women in the greater Houston area and their health care providers about breast cancer, and to compare patients' and health care providers' perceptions of barriers to breast cancer screening. To quantify these measures, we administered a brief questionnaire both prior to and following the educational seminars.

Prior to the seminar, health care providers were moderately knowledgeable about breast cancer risk factors and screening recommendations, but were confused about the implications of hereditary predisposition. Patients, on the other hand, had little knowledge of breast cancer risk factors, screening guidelines or hereditary predisposition to breast cancer. Following the educational seminar, both health care providers and patients showed significant improvement in knowledge, however, the concept of risk remained difficult for both groups.

Given the enthusiasm with which the seminar was received, as well as the formidable improvements in knowledge made by the participants, we propose suggestions for modification of the program for the purpose of continued implementation.

Program Nr: 2194 from the 1999 ASHG Annual Meeting

Breakpoints are clustered on chromsome 2 [p11q13] resulting in pericentric inversion. *R.S. Verma[1,2,3], S.M. Kleyman[1], D.A. Rosa[4], S.K. Barrett[2,4], G.S. Kupchik[2,4].* 1) Institute of Biology and Genetics at InterScience, Brooklyn, N.Y; 2) SUNY Health Science Center at Brooklyn, N.Y; 3) Wyckoff Heights Medical Center, Brooklyn/New York Hospital-Weill Medical College of Cornell University, New York, N.Y; 4) Maimonides Medical Center, Brooklyn, N.Y.

Except increased frequencies of reproductive wastage among carriers of a pericentric inversion of chromosome 2, no apparent risk to liveborns have been reported. In most cases the breakpoints on chromosome 2 have been clustered within bands 2p11 and 2q13. However, in a few cases the breakpoints have been noted on p12, p13, p14, p15, p21, p23, p24, p25 and q11, q12, q14, q21, q23 and q36. With a few exceptions, if the breakpoint is in the short arm in band 2p11, the band on the long arm involved was 2q13. A couple was referred for cytogenetic evaluation because they have had 2 ELAB, 1 SPAB plus one prior ELAB with a different partner, while the fourth pregnancy was terminated because of abnormal cytogenetic findings [48,XXX,+18] in amniocytes. The fifth pregnancy again, resulted in fetal loss. Cytogenetic findings with G-banding revealed an abnormal karyotype, 46,XY,inv(2)(p11q13) in the husband's peripheral blood while the wife has a very low level of mosaicism for the X-chromosome i.e. 46,XX[49]/47,XXX[1]. This fortuitous finding of low level mosaicism can not be correlated with double trisomy but increased risk of aneuploidy needs to be mentioned. There is a 10% risk of multiple congential anomalies/mental retardation to the infant if the origin of such inversions are *de novo* but no apparent risk has been cited for inherited cases. Earlier cases with pericentric inversion of chromosome 2 are being reviewed and the counseling aspects discussed.

Program Nr: 2195 from the 1999 ASHG Annual Meeting

Segregation analysis of inattention and hyperactivity-impulsivity. *B.S. Maher[1,2], M.M. Vanyukov[1,3,5], M.L. Marazita[1,2,3,4].* 1) Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA; 2) Cleft-Palate Craniofacial Center, School of Dental Medicine, Univ. of Pittsburgh, Pittsburgh, PA; 3) Center for Education and Drug Abuse Research, Univ. of Pittsburgh, Pittsburgh, PA; 4) Department of Oral and Maxillofacial Surgery, School of Dental Medicine, Univ. of Pittsburgh, Pittsburgh, PA; 5) Department of Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA.

Segregation analysis was performed on 602 nuclear families in an attempt to estimate the role of genetic and other influences in determining the variability of inattention and hyperactivity-impulsivity(H-I), the behavioral dimensions underlying Attention Deficit Hyperactivity Disorder (ADHD). Families for our study were ascertained as part of a larger study of substance abuse (SA) risk. Factor analysis of the 14 DSM-III-R ADHD symptoms revealed two distinct factors. Items loading on the first factor consisted of the six inattention symptoms. The second factor contained the eight hyperactivity and impulsivity symptoms. Two phenotypes for each individual were determined by summing the items loading on each factor. Segregation analysis was performed separately for inattention and H-I dimensions in the complete data set and the high (309 nuclear families) and average (293 nuclear families) SA risk subgroups. Class D regressive models with covariate effects (SES) and sex dependent means were tested. Segregation analysis of the inattention factor supported a sex-dependent Mendelian codominant model in the complete data set ($p=0.7473$) and in each of the subgroups(high risk: $p=0.2557$; average risk: $p=0.6865$), while other models could be rejected. Segregation analysis of the H-I factor supported a transmissible but non-Mendelian major effect in the complete data set and in each of the SA risk subgroups. Thus, this study demonstrates Mendelian transmission of DSM-III-R defined inattention symptom count and non-Mendelian transmission of hyperactivity-impulsivity in a general population. Overall,our results support the presence of a two distinct endophenotypes (inattention and hyperactivity/impulsivity) underlying the liability to Attention Deficit Hyperactivity Disorder.

Program Nr: 2196 from the 1999 ASHG Annual Meeting

Latitudinal Trends Among Native North American Groups: Examination of Mitochondrial DNA Diversity. *R.S. Malhi[1], B.A. Schultz[1], J.A. Eshleman[1], J. Peterson[1], J.G. Lorenz[2], D.G. Smith[1]*. 1) Dept Anthropology, Univ California, Davis, Davis, CA; 2) National Institues of Health, NIAAA/DICBR/LNG.

Native North American groups display a non-random distribution of haplogroup frequencies across regional and linguistic boundaries. We analyzed the mitochondrial DNA (mtDNA) haplogroup frequency distribution and the mtDNA Control Region (CR) diversity among and within Siouan, Iroquoian, and Algonquian language groups that span from the Rocky Mountains to the Eastern Seaboard. The homogeneity of haplogroup frequency distributions among linguistically related Native American groups located in different geographic regions suggests that ancestral groups are similar in haplogroup frequency distribution to descendant groups. This is consistent with increasing evidence, from studies of ancient mtDNA, that genetic drift has not led to significant stochastic changes in frequencies that would obscure ancestor/descendant relationships. Analysis of mtDNA CR sequences also reveals trans-continental trends among these Native American groups. These trends, along with linguistic and archaeological data, suggests significant movement and tribalization of Native American groups in North American prehistory.

Program Nr: 2197 from the 1999 ASHG Annual Meeting

The unstable mutations on the mitochondrial haplogroup T background. *B. Malyarchuk, M. Derenko.* Genetics Lab, Biological Probl of the North, Magadan, Russia.

The utility of mtDNA markers for study of prehistoric female migrations from one geographic region to another requires the plausible classification for mitochondrial variations. The classification system based on cladistic notation for mtDNA hypervariable segments sequences and RFLPs is developed for West Eurasian mtDNAs and provides motifs, or signature mutations, which are diagnostic for mtDNA haplogroups (Richards et al., 1998). However, the problem of homoplasy at nucleotide sites in mtDNA, especially in the hypervariable segment-1 (HVS-1), remains actual. Haplogroup T with motif 16126C-16294T is one of the most frequent among Europeans. It has been shown that this haplogroup includes the only well-resolved subgroup T1, but other HVS-1 sequences cannot be classified in subgroups due to the possible homoplasy at sites in positions 16292, 16296, and 16304, leading to the reticulations in the topology of phylogenetic networks. To study the problem of molecular instability at these nucleotide positions, we have performed an analysis of published data sets and our unpublished data on mtDNA HVS-1 sequences in Eastern Slavonic sample. After haplogroup T HVS-1 sequences were combined, 172 individual mtDNA sequences were obtained. As a principal analytical approach, a search for a rear mutations, associated with different combinations of nucleotides 16292T, 16296T and 16304C within the haplogroup T sequences and specific to certain ethnic group or the group of closely related by descent populations, was performed. The analysis revealed ten marker mutations, each of them is characteristic for certain group of linguistically or geographically close individuals - for the Adygei, Germans, Kazakhs, and linguistic isolates of the eastern Italian Alps. The occurrence of these marker mutations in association with various combinations of mutations at positions 16292, 16296 and 16304 gives the evidence of molecular instability at positions 16292 and 16296 on the haplogroup T background, at least. The data received allow us to conclude that the identical haplogroup T sequence types might have arisen independently in different ethnic groups.

Program Nr: 2198 from the 1999 ASHG Annual Meeting

Comparison of the Type I error rate of model-dependent linkage analysis for quantitative traits under random ascertainment. *D.M. Mandal[1], A.F. Wilson[2], A.J.M. Sorant[2], B.J.B. Keats[1], J.E. Bailey-Wilson[2].* 1) LSU Medical Center, New Orleans, LA; 2) National Human Genome Research Institute, NIH, Baltimore, MD.

The Type I error rate for model-dependent lod-score linkage analysis may be increased when marker allele frequencies are misspecified and ascertainment is through the trait. When ascertainment is random, as is often the case in linkage studies of quantitative traits, lod-score methods are expected to be robust. However, when parental marker data are missing, lod-score linkage analysis of a trait with a single-locus heritability of 90% is not robust to misspecified marker allele frequencies, even in large random samples of sib-pairs. In the present study, the Type I error rate of the lod-score linkage method was determined for additional traits in the absence of parental data and with misspecification of marker allele frequencies.

G.A.S.P. was used to generate a highly polymorphic marker locus with varying marker allele frequencies and two quantitative traits unlinked to the marker: one 50% due to an additive major locus with two equifrequent alleles and the other one due completely to environmental effect. LODLINK was used to test for linkage in each of 10,000 replicates of 300 families with sibship size two. When the trait was completely environmentally determined, the Type I error rate of the lod-score linkage analysis was found to be robust to misspecification of marker allele frequencies, even if the allele frequencies were severely misspecified (±0.8 of true frequencies). However, for the trait with heritability of 50%, the Type I error rate was sometimes as much as 21 times the nominal significance level of 0.001 when the allele frequency of a common allele in a five-allele polymorphic marker was misspecified in the analysis as rare. In general, the Type I error rates for the trait with a heritability of 90% were the highest among the three traits studied. Thus, when the parental genotype data are missing, the model-based lod-score test of linkage is robust with respect to Type I error for environmentally determined traits but not for the traits which are largely genetically determined.

Program Nr: 2199 from the 1999 ASHG Annual Meeting

The human twinning gene is not syntenic with the sheep twinning gene (FecB) on human chromosome 4. *N.G. Martin[1], D.L. Duffy[1], J. Hall[2], C. Mayne[1], S. Healey[1], J. Brown[3], G.W. Montgomery[1,3], D.I. Boomsma[4].* 1) Queensland Inst Medical Res, Brisbane, QLD, Australia; 2) AxyS Pharmaceuticals Inc, La Jolla, CA; 3) Univeristy of Otago, Dunedin, New Zealand; 4) Vrije Universiteit, Amsterdam, Netherlands.

The tendency to dizygotic twinning is inherited in both humans and sheep. The fecundity gene in Booroola Merino sheep, FecB, has been mapped to sheep chromosome 6 syntenic with human 4q21-25. Our aim was to see whether a gene predisposing to human DZ twinning can be mapped syntenic with FecB. DNA was collected from 169 pairs and 17 sets of 3 sisters (trios) from Australia and NZ who had each had spontaneous DZ twins, mostly before the age of 35, and from a replication sample of 111 families (92 affected sister pairs) from the Netherlands. Exclusion mapping was carried out after typing 26 markers on chromosome 4, of which 9 spanned the 4q21-25 region. We used nonparametric affected sib pair methods for linkage analysis (ASPEX 2.2). Complete exclusion of linkage (lod < -2) of a gene conferring relative risk as low as 1.5 (lambda(S) >1.5) was obtained for all but the p terminus region on chromosome 4. Exclusion in the syntenic region was stronger, down to lambda(S)=1.3. We conclude that if there is a gene influencing DZ twinning on chromosome 4, its effect must be minor. We are currently completing a genome scan and have several regions showing linkage with lods of 2.3, 1.3 and 1.2 which we are pursuing.

Program Nr: 2200 from the 1999 ASHG Annual Meeting

Nucleotide diversity levels at the human alpha-fetoprotein gene AFP1. *J.J. Martinson[1], R.E. Ferrell[2].* 1) Dept Biologicial Anthropology, Oxford University, Oxford, England; 2) Dept Human Genetics, University of Pittsburgh, Pittsburgh PA 15261.

The development of automated sequencing technologies has greatly facilitated the generation of large amounts of sequence data in human populations. Here we report initial data from a survey of sequence variation at the AFP1 gene complex, located on chromosome 4. This gene was chosen for analysis as it is known to be highly variable between primate species, but little is known about its intraspecific variation within humans. The gene product has many functions, but these are poorly understood.

The AFP gene occupies 27kb of DNA and contains several repeat elements in addition to the 2kb of coding sequence that encodes the mature protein. These include Alu, L1, STR and some novel repeat elements. In a sample of 96 unrelated individuals, none of these, with the exception of the STR loci, were found to be polymorphic for repeat number polymorphism. We have begun a study on sequence variation at this gene, and have generated data from 34 unrelated individuals for a region encompassing exon 4 and its flanking introns, which contain a Kpn element and an Alu repeat. To date, over 67kb of sequence data have been generated. Each individual was sequenced in both directions to confirm the polymorphisms seen.

The levels of sequence diversity seen here are comparable with results obtained from studies on other loci. In the 2kb region studied, 15 segregating sites were seen, of which 8 were singletons. No insertion/deletion polymorphisms were observed. Nucleotide diversity levels p were 0.047% for the Kpn repeat region, 0.058% for exon 04, and 0.091% for the Alu repeat region. This preliminary survey would suggest that the Kpn element is not associated with elevated levels of flanking sequence variation, but that diversity is higher in the region around the Alu repeat.

Program Nr: 2201 from the 1999 ASHG Annual Meeting

DYNAMICS OF MOLECULAR GENETIC DIVERSITY IN THE EAST MIDLANDS, ENGLAND. *S.S. Mastana, D.R. Lee.* Dept Human Sci, Human Gen Lab, Loughborough Univ, Loughborough, England.

The main objectives of this investigation were 1) to establish the database of minisatellites (VNTRs), AMP-FLPs, microsatellites (STRs), and ALU Insertion allele frequencies for the regionally sub-divided populations of the East Midlands, which is suitable for population genetic and forensic investigation, 2) to determine if Caucasian sub-population heterogeneity exits within the United Kingdom, within Europe and World Caucasian and racial populations at these loci and 3), whether settlement patterns of various continental European populations have left any detectable genetic imprints in the East Midlands populations. Blood samples (500) were collected at random from the Caucasian East Midlands populations (5 sub-populations, Northwest Derbyshire, Northeast Derbyshire, South Derbyshire, Leicestershire and Nottinghamshire). Using standard molecular genetic techniques, we analysed MS1, MS31, YNH24, MS43a VNTRs and HUMTHO1, F13A, F13B, FES, LPL, VWA31 and CSF1PO STRs. Alu insertion polymorphisms studied included, ACE, TPA, PV92, APO and FXIIIB and three AMP-FLPs were D1S80, APO-B, and YNZ22. Overall significant differences were observed at many different loci. While overall pattern of allelic distribution was within the ranges observed for Caucasian populations, there were significant inter-population/regional differences for a number of loci. As expected the heterozygosity levels for DNA loci were much higher than conventional blood groups. The F_{ST}/G_{ST} values were also higher for DNA loci (average 0.018) compared to blood groups and serum proteins (0.005). The implications of observed genetic diversity in urban contemporary populations are evaluated in the light of settlement patterns of continental European populations. The Caucasian population heterogenetity and its implications for disease mapping, forensic and paternity investigations are presented to evaluate the effectiveness of these markers.

Program Nr: 2202 from the 1999 ASHG Annual Meeting

A net avoidance of close consanguinity on Tangier Island, Virginia. *R.A. Mathias[1], C.A. Bickel[1], A. Shahani[2], T.H. Beaty[1], K.C. Barnes[1].* 1) Johns Hopkins University, Baltimore, MD; 2) George Washington University, Washington, DC.

In small isolated populations mates tend to be less related than two individuals picked at random due to social proscriptions against close consanguinity; thus, a heterozygote excess resulting from avoidance of incestuous mating can be expected. We assessed the presence and magnitude of this heterozygote excess on Tangier Island, Virginia using genealogic data dating back to 1722. With a present population of ~650 permanent residents, we have observed low levels of average inbreeding (F=0.006) in the present generation, which peaked at F=0.01 in 1850 and 1900. To test for heterozygote excess, observed inbreeding was compared to expected inbreeding over 25-year intervals from 1800-1998. At each time point the population was divided into a parent generation and an offspring generation. Observed inbreeding was computed by averaging the inbreeding coefficients for all offspring. Expected inbreeding was computed by averaging kinship coeffieients for (a) all male-female pairs; (b) non-sib male-female pairs; and (c) first-cousin and closer male-female pairs exlcuded in the parent generation. All expected levels of inbreeding exceeded observed levels in the offspring generation between 1.19-11.89 fold. The greatest differences were in the earlier birth cohorts where the parental generation tended to be small and the avoidance of close consanguinity most prominent. We also examined a coefficient d to measure the deviation from random mating. This d, which represents a correlation between uniting gametes relative to the population's genotypic structure, tends to be negative when there is an avoidance of mating of close relatives. Values of d were computed for all three types of parental pairs, and were found to be negative at all time points (-0.001 to -0.027), with earlier cohorts having the lowest values. While recognizing that several factors influence the genotypic structure of a population, we speculate that the decreasing trends in heterozygote excess observed in this extended kindred is a reflection of an increase in mate choices accompanied by a breakdown in isolation and a shift towards exogamy.

Program Nr: 2203 from the 1999 ASHG Annual Meeting

Age-related differences in heterozygote frequencies of mutations commonly found in the Ashkenazi Jewish population. *L.D. McCurdy[1], B. Breuer[2], L. Lebow[2], J. Bernstein[1], R.J. Desnick[1], C.M. Eng[1].* 1) Mount Sinai School of Medicine, New York, NY; 2) The Jewish Home and Hospital, New York, NY.

The Ashkenazi Jewish population is at increased risk for several autosomal recessive conditions. In addition, common mutations in the BRCA1, BRCA2, and APC genes have been identified. Carrier frequencies for these mutations have been determined mainly from Ashkenazi Jewish individuals of reproductive age. In an effort to discern if age-related differences in carrier frequencies exist for any of these mutations, an elderly cohort of 714 Ashkenazi Jewish individuals (avg age 80 yrs;572 females/142 males) was assayed for the following: N370S(GD), delATCTGA/insTAGATTC (BLM), I1307K(APC), 185delAG, 5382insC, and 6174delT (BRCA1&2).

mutation	observed	expected
N370S	31 (4.3%)	37 (5.2%)
delATCTGA/insTAGATTC	4 (0.6%)	6.4 (0.9%)
I1307K	41 (5.7%)	43 (6%)
185delAG,5382insC,6174delT	3 (0.4%)	15 (2%)

Observed rates for the Gaucher disease (GD), Bloom syndrome (BLM), and APC mutations was not significantly different from expected. However, among 142 males no N370S heterozygotes were observed while seven were expected (p=0.014). No difference in I1307K carriers was observed, suggesting that heterozygotes for this variant are not at increased risk. In contrast, female heterozygotes for the BRCA1 and BRCA2 mutations was significantly less than expected (p=0.012). These results indicate that female BRCA1 and BRCA2 carriers are underrepresented in our elderly Ashkenazi Jewish population, suggesting that these mutations may have a relatively higher age-related risk.

Program Nr: 2204 from the 1999 ASHG Annual Meeting

Comparison of several methods to calculate sample size for TDT genome scans. *R.E. McGinnis.* Dept. of Human Genetics, SmithKline Beecham, Harlow, England.

Recently, Tu and Whittemore (AJHG 64:641, 1999) and Knapp (AJHG 64:1777, 1999) presented equations for calculating number (N) of affected sib pair families required for genome scans with the TDT. Both methods avoid simplifying assumptions such as independence of allele transmissions from mother and father to affected offspring in order to calculate precise sample sizes; but the required algebraic formulations are very complex. Like these authors, I also calculated N needed for 80% power to detect linkage with a 500,000 marker TDT scan, but used the following equation for two methods that retain simplifying assumptions: $N=(Z_{a/2}-YZ_{1-b})/[(H/F)(4P_t-2)^2]$. Here Y equals $[1-(H/F)(2P_t-1)^2]^{1/2}$ for method 1 (a generalization of the approach of Risch and Merikangas [Science 273:1516, 1996]) while Y equals $2[P_t(1-P_t)]^{1/2}$ for method 2 (described by McGinnis in Ann Hum Genet 62:159, 1998). For both methods, expressions for P_t and H/F are given in McGinnis (1998) where P_t is the probability of marker allele transmission from a heterozygous parent to an affected child and H/F is the probability that an ascertained parent is heterozygous. Results for methods 1 and 2 were compared with the methods of Knapp and of Tu and Whittmore for the 48 disease models listed in Table 4 of Knapp (1999). For each pairwise comparison between two methods, the table below shows the mean percent difference in N for the 48 models ±StdDev, and the largest percent difference observed (in parenthesis). These comparisons indicate that sample sizes calculated by the four methods do not differ substantially.

	Tu & Whittemore	Method 1	Method 2
Knapp	4.1%±4.0% (16%)	0.7%±0.7% (3%)	1.0%±0.9% (3%)
Tu & Whittemore	---	3.7%±3.8% (16%)	4.8%±4.7% (17%)
Method 1	---	---	1.2%±1.4% (5%)

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Program Nr: 2205 from the 1999 ASHG Annual Meeting

Towards strategies to assess genetic risk of complex disorders in a multi-ethnic community: ACE polymorphism and diastolic blood pressure in pregnancy. *I.Y. Millwood, E.C. Winchester, M.A. Penny, L. Rand, O.J. Fletcher, A.M. Kessling.* Medical and Community Genetics, Imperial College School of Medicine, London, UK.

Morbidity and mortality from complex genetic disorders may be reduced if genetic screening programs can identify susceptible individuals before disease development. Genetic risk factors are likely to vary between population groups and screening programs would need to cater for the different genetic backgrounds present within communities. In working towards screening tests for polygenic disease risk, we investigated the effects of three genetic variants on diastolic blood pressure variability in a local sample of healthy pregnant women (n=1311) from different genetic backgrounds. The sample included two main population groups, women of UK and Irish origin (n=537), and those with ancestors from the Indian subcontinent (India, Sri Lanka, Pakistan, Bangladesh) (n=391). Individuals were genotyped for known polymorphic variants of the ACE, AGT and AGTR1 genes, which have been previously associated with hypertension. Allele frequencies in UK+Irish and Indian subcontinent groups respectively were ACE I/D 0.45/0.55 and 0.57/0.43, AGT T174M 0.90/0.10 and 0.88/0.12, and AGTR1 A1166C 0.71/0.29 and 0.93/0.07. There were significant differences in genotype distribution between the UK+Irish and Indian subcontinent groups for the ACE I/D and AGTR1 A1166C variants ($p<0.001$), but not for AGT T174M. ACE genotype had a small but consistent influence on basal diastolic blood pressure in all population groups of our sample, the ACE D allele coinciding consistently with increased blood pressure. AGT and AGTR1 genotype did not influence blood pressure consistently across population groups. Our data show that combining samples from different populations may mask population specific effects. Future tests for polygenic disease risk must combine information from multiple genes and take into account population diversity.

Program Nr: 2206 from the 1999 ASHG Annual Meeting

Differences in nutritional pattern between mothers of children with cleft lip and palate and mothers on non-affected children. *J. Mir[1], T. Mosby[1], S. Tolarova[2], L. Pastor[3], A. Goldberg[4], M. Pastor[3], A. Capozzi[5], M. Tolarova[1].*
1) Program for Prevention of Cleft Lip and Palate/Craniofacial Anomalies, UCSF, San Francisco, CA; 2) Veteran's Hospital, University of Minneapolis,MN; 3) University of Cuyo, Mendoza, Argentina; 4) Marin General Hospital, San Rafael, CA; 5) Shreiners Hospital, Sacramento, CA.

Orofacial clefts(OFC)are common and serious birth defects with a prevalence of 1.8/1,000 births.The majority of OFC are caused by interaction of genetic and environmental factors. However,the specific nature of this interaction remains poorly understood.Mother's diet and life style during the embryonic period seems to contribute significantly to environmental factors.There is no doubt that folic acid(FA)plays a key role in etiology of NTD,and very probably in OFC and other dysraphic anomalies.However,still many questions remain.In OFC specifically,the question of the dose of folic acid that is necessary to reduce recurrence and the dose needed for prevention of occurrences still needs to be answered.Our present case-control study is based on an analysis of a sample of 111 mothers of children with OFC and 73 mothers of nonaffected children from Cumana,Venezuela.A personal interview including detailed demographic data,information about living conditions,size and socio-economic status,pregnancy and labor history,mother's and father's medical history, etc.,was conducted with each mother.Cases and controls came from rural areas and belong to a low socio-economic category.Most interesting results were seen when evaluation of consumption of vegetables and fruit was carried out.OFC mothers did consumed practically no fresh vegetables.Only 1.9% of case mothers reported consumption of fresh vegetables one or more times per day. The majority consumed cooked vegetables (74.29% of case mothers vs.32.95%control mothers).Also consumption of fresh fruit in the group of case mothers was minimal.We can conclude that OFC mothers very likely did not have enough FA from the diet during their pregnancies and this could contribute to development OFC. This study was initiated by the Program for Cleft Prevention UCSF and supported by Rotaplast Intl., Inc.

Program Nr: 2207 from the 1999 ASHG Annual Meeting

Genetic association using affected siblings: application to inflammatory bowel disease (IBD) and the HLA-DRB1*0103 gene. *L. Mirea[1], S.B. Bull[1], M.S. Silverberg[2], A.H. Steinhart[2], R.S. McLeod[2], A. Griffiths[4], G.R. Greenberg[2], Z. Cohen[2], J.A. Wade[3], K.A. Siminovitch[1,2]*. 1) Samuel Lunenfeld Research Institute, University of Toronto, Toronto, ON, Canada; 2) Mount Sinai Hospital, Toronto, ON, Canada; 3) The Toronto Hospital Regional Histocompatibility Laboratory, Toronto, ON, Canada; 4) The Hospital for Sick Children, Toronto, ON, Canada.

Standard methods for genetic association analysis, including population case-control and parental control transmission disequilibrium tests (TDT) may be sensitive to lack of independence amongst cases. Association studies are often conducted using affected sibpair (ASP) family data collected for genome scans with independence achieved by randomly selecting one sibling. This process, however, discards at least half of the available cases. The methodology applied in this study permits the use of affected sibpairs in both case-control and TDT analyses. Association between IBD and HLA DRB1*0103, was tested in 216 healthy controls and 120 IBD ASP families using a logistic regression model and the TDTEX procedures of SAGE. Analyses were first conducted with independent randomly selected cases and then using affected siblings assumed to be independent. A generalized estimating equations (GEE) approach, that provides robust variance estimates, was applied to the logistic regression model to adjust for the correlation among siblings. The SIBS TDTEX option of SAGE was employed to assess preferential allelic transmission to the sibpair as a unit. Both case-control (assumed independent: p=0.0005, adjusted: p=0.0011) and TDT (assumed independent: p=0.00003, adjusted: p=0.0039) analyses of this data set confirm that the risk of DRB1*0103 is estimated more conservatively when a correction for sibling correlation is applied than when naïve analysis assumes siblings are independent. More importantly, the risk estimated using proper correlation adjustments with GEE (independent cases: p=0.0026) and SIBS TDT (independent cases: p=0.01946) provides stronger evidence for association than results obtained from analysis of only independent cases.

Program Nr: 2208 from the 1999 ASHG Annual Meeting

ATM Haplotypes in Hispanic Ataxia-Telangiectasia (A-T) families. *E.M. Mitui[1], S. Castellvi-Bel[2], R. Gatti[1].* 1) Department of Pathology, School of Medicine, UCLA, Los Angeles, California; 2) Servei de Genetica, Hospital Clinic i Provincial, Barcelona, Spain - As well as collaborators from each of the countries cited.

This study investigates the possibility of Hispanic A-T patients from different geographic areas sharing a common haplotype and mutation. Initially, we are characterizing A-T patients from Spain (18), Brazil (10) and Argentina (10), using four polymorphic microsatellite markers (D11S1819, NS22, D11S2179 and D11S1818) in an interval spanning approximately 1.4 centiMorgans, within and flanking the ATM gene. All alleles are compared to a standardized sample (CEPH 1347-2). We present the haplotype results of 38 A-T affected patients from 31 families, 16 families from Spain, 7 families from Brazil and 8 families from Argentina. These haplotypes are being compared to 54 Costa Rican families carrying seven mutations and 17 Hispanic American families. The haplotype analysis described here will be extended by identifying the mutations present on each unique haplotype within each ethnic group. To date, no founder effect haplotype has been convincingly documented between these five Hispanic groups.

Program Nr: 2209 from the 1999 ASHG Annual Meeting

Languages, geography and HLA haplotypes in Native American and Asian populations. *M.V. Monsalve[1,2], A. Helgason[3], D.V. Devine[2]*. 1) Dept of Anthropology and Sociology, Univ of British Columbia, Vancouver, BC, Canada; 2) Dept of Pathology, University of British Columbia, Vanciuver, BC, Canada; 3) Department of Biological Anthropology, University of Oxford, UK.

A number of studies based on linguistic, dental and genetic data have proposed that the Amerindians, Na-Dene and Eskimo-Aluet are the descendants of three waves of migration from Northeast Asia to the New World. Recently other studies have suggested that only one major migration occurred. It is our aim in this study to test these opposing migration hypotheses using molecular typed HLA class II alleles to compare the relationships between linguistic and genetic data in contemporary Native American populations. The second exons of the DRB1, DQB1 and DQA1 were amplified in Salishans belonging to the Amerindian linguistic group from the Soawahlie band in British Columbia, Canada. The detection of the alleles were typed using non-radioactive sequence specific oligonucleotides. Haplotype frequencies were estimated using the maximum likelihood method. DRB1/DQB1/DQA1 haplotype frequencies were obtained from 20 Native American groups and 11 Asian populations. Our results suggest that gene-flow and genetic drift have been important factors in shaping the landscape of the Native American populations. Our results also indicate significant correlations between genetic and geographic distances in Native American and East Asian populations. In contrast, a less clear-cut relationship seems to exist between genetic distances and linguistic affiliation. In particular, the close genetic relationship of the neighboring Na-Dene Athabaskans and Amerindian Salishans suggests that geography may be the more important factor. Overall, our results are most congruent with the single migration model.

Program Nr: 2210 from the 1999 ASHG Annual Meeting

Environmental and nutritional factors in etiology of cleft lip and palate anomalies in Guanajuato, Mexico. *T. Mosby[1], A. Goldberg[2], J. Mir[1], J.A. de la Peña[3], M. Covarrubias[3], M. Chavez[4], J.M. Ventura[4], M. Tolarova[1]*. 1) Program for Prevention of Cleft Lip and Palate/Craniofacial Anomalies, UCSF,San Francisco, CA; 2) Marin General Hospital,San Rafael, CA; 3) Programa Nuevo Amacener, Guanajuato,Mexico; 4) Fundacion Mexicana para la Prevencion de Malformaciones Craneofaciales,Leon, Mexico.

Cleft lip and palate affect 1 in 580 Hispanic newborns.Non-syndromic clefts(NSC)are caused by a combination of genetic and environmental factors; the nutrition represents an important part of the environmental ones.As a part of a large case-control study on gene-environmental interactions in NSC,a pilot baseline nutritional study was carried out in Guanajuato,Mexico.Data were obtained from interviews of 118 mothers of NSC child.The interview instruments consisted of 152 questions from General and Food Frequency Questionnaire.75%of our families came from a low or very low socio-economic class.70% of mothers were housewives;5% had occupational exposure to toxic chemicals.A base of the Food Pyramid consisted in all familes of tortillas and beans.Raw vegetables were eaten sporadically (51%)or not at all (23%),but 65% of mothers consumed meat 1-3 times per week.Among our mothers,alcohol,drugs and tobacco use were rare;diabetes and obesity were high.A low level of red cell folate(21 mothers,199.14±15.5 nM/L)was found.Normal range of plasma folate and vitamin B12(32 mothers,24 patients,21 controls)were found(a meal was provided in the hospital!).This pilot study showed that the mothers"nutrition was very inadequate, especially consumption of raw vegetables and fresh fruit was very low.Thus,nutritional factors could contribute to the causes of NSC in the Guanajuato state.Public measures such as fortification of staple food with essential nutrients, recommendation of life style and cooking changes and a broad educational campaign based on present knowledge could also help to decrease the risk for clefting,if implemented. This study was initiated by the Prg.for Prev.of Cleft Lip and Palate/Craniofac.Anom.,UCSF,USA; funded by the Government of Guanajuato,Mexico, supported by Programa Nuevo Amanecero,and Fundac.Mex.para la Prev.de Malf.Craneofac.A.C.

Program Nr: 2211 from the 1999 ASHG Annual Meeting

Sample size requirements for detecting a genotype associated with an adverse drug reaction. *M. Mosteller.* Clinical Genetics Division, Glaxo Wellcome Research & Development, Research Triangle Park, NC.

Each year, hundreds of thousands of patients suffer adverse drug reactions (ADRs). These ADRs can exact a personal toll ranging from simple discomfort to significant injury or even death. The economic impact of treatments, hospitalizations, and lost productivity only add to the importance of finding ways to reduce the occurrence of ADRs. One strategy toward this end is to identify drug-specific patient genotypes that are correlated with the occurrence of ADRs. Such genotypes could be used to ensure that a given drug is prescribed only for individuals with a low risk of experiencing an ADR. A first step in this pharmacogenetic strategy is to determine the number of clinical trial subjects that would be required to demonstrate a statistical association between a genotype and an ADR. Computer simulation was used to investigate this issue under a variety of hypothetical conditions. Given an ADR whose expression is influenced by a single genetic locus, the impact of the following parameters on sample size was explored: prevalence of the ADR, allele frequencies at the causative locus, genotypic penetrance, mode of inheritance, phenocopy rate, degree of linkage disequilibrium with a marker locus, statistical significance level, and desired statistical power. Under conditions that can be considered plausible, the number of subjects required was estimated to be in the hundreds rather than in the thousands. In practice, the number of subjects needed could be arbitrarily large due to low penetrance, high phenocopy rate, or low level of linkage disequilibrium between the causative and marker loci. This work indicates that the search for a patient genotype that is predictive of an ADR may require less than 100 subjects or well over 500. Progress in this area will likely require combining information gathered across several clinical trials and, until tools and technologies emerge that will support genome-wide association studies based on high density maps, will be highly dependent on the selection of appropriate candidate genes and markers.

Program Nr: 2212 from the 1999 ASHG Annual Meeting

Methylenetetrahydrofolate reductase C677T gene mutation, embryo-maternal genotypes interaction, maternal periconceptional folic acid intake and neural tube defects in the Mexican population. *O.M. Mutchinick, M.A. Lopez, L. Luna, J. Waxman, V. Babinsky, RYVEMCE Collaborative Group.* Dept of Genetics, Inst Nac de Nutricion Salvador Zubiran, Mexico,D.F., E-mail: osvaldo@servidor.unam.mx.

Neural tube defects (NTD) and the C677T MTHFR gene mutation have a very high prevalence in the Mexican population, nearly 3 to 4 times higher than in other ethnically related groups. According to our own data (RYVEMCE), 4/1000 births have a NTD, 34.8% of normal individuals are homozygous for the mutation, and Mexican embryos have a higher a priori risk (OR 5.8%) of being carriers of the thermolabile variant (48th ASHG Meeting, A302). This genetic condition is considered a risk factor for anencephaly and spina bifida.The above prompted us to develop a multicentric study to investigate the prevalence of the expected CC, CT and TT genotypes in a sample of 171 cases of NTD, 171 matched controls and their respective mothers. Periconceptional maternal folic acid (FA) and other vitamins intake was estimated using a food frequency questionnaire validated for our population. DNA studies of the 682 blood samples obtained were performed as previously described (48th ASHG Meeting, A302). Our results showed a significantly higher prevalence of the TT genotype in NTD cases (35.7%) than in controls (23.4%), OR:1.81 (1.1-3.0), p: 0.018, with an attributable fraction (AF) for the homozygote TT of 16.03 %. No statistical differences were observed between mothers. The analysis of embryo-maternal genotypes (TT/TT) interaction between cases (25.9%) and controls (15.3%), also showed significant statistical differences, OR: 1.93 (1.1-3.5), p: 0.02, with an AF of 12.5% for that genotypic array. FA daily intake by the mothers of cases was lower than that of control mothers. This difference was even higher when mothers of TT cases (median 168.8ug), were compared with mothers of TT controls (median 235.5ug). These results strongly suggest that a considerable proportion of the very high prevalence of NTD in our population can be explained by the high prevalence of the homozygote for the MTHFR C677T mutation and the associated low maternal folic acid intake during the periconceptional period.(CONACYT, grant # MP0936-96).

Program Nr: 2213 from the 1999 ASHG Annual Meeting

Estimation of the Frequency of Genetic Deafness in Mongolia with a Genotype Index. *W.E. Nance[1], R. Erdenetungalag[2], J. Radnaabazar[2], B. Dangaasuren[3], J. Batsuuri[4], S.H. Blanton[1], W.H. Carter[1], A. Pandya[1].* 1) Dept Human Genetics, Medical Col Virginia, Richmond, VA; 2) MCH Research Center; 3) School of the Deaf; 4) Center for Anthropology, Ulaanbaatar, Mongolia.

Estimates of the genetic component of deafness are based on the recognition of syndromic forms and the assumption that multiplex non-syndromic families are also genetic. Extrapolating from the distribution of affected individuals in multiplex non-syndromic families, the proportion of simplex families that are chance isolated genetic cases can be estimated by segregation analysis. However, it is difficult to appropriately allow for heterogeneity in the penetrance and mode of inheritance for the multiple genes for deafness that are now known to exist. The recognition that mutations at some loci including Connexin 26 and mitochondrial forms of deafness have a high prevalence in many populations suggests that a comparison of frequency of these entities in multiplex and simplex families could be used as an index to estimate the overall frequency of genetic deafness using the formula:

where M and (N-M) are the overall numbers of multiplex and simplex probands and I_s & I_m the number positive for the index trait (s) among n tested mulitplex and simplex probands respectively. To test this approach, we studied 533 school aged deaf Mongolian probands including 12 with recognized forms of syndromic deafness. This sample, along with the deaf relatives of affected probands is estimated to include approximately 70% of the school aged deaf population of Mongolia. The non-syndromic probands included 112 multiplex and 409 simplex families. Twenty of the multiplex and 15 of the simplex probands carried mutations at the mt 1555 site or the mt 7445 restriction site respectively. The overall incidence of mitochondrial deafness in the 533 probands was 6.5%. The estimated proportion of genetic cases among the non- syndromic probands was 37.6 ± 5.8% with an overall estimated frequency of genetic deafness of 39.0%.

Program Nr: 2214 from the 1999 ASHG Annual Meeting

Prevalence of Factor V R506Q in the newborn population of Northwest Louisiana. *D. Napper, K. Yanamandra, D.W. Jalanivich III, T.F. Thurmon, H. Chen, M. Susla, M. Jeroudi.* Dept Pediatrics, Louisiana State Univ Med Ctr, Shreveport, LA.

The Clinical Molecular Genetics Laboratory at LSU Medical School in Shreveport, Louisiana, has been studying the genetics of Activated Protein C resistance (APCR) by assaying mutant Factor V arg506gln.

The mechanism of thrombophilia especially APCR causing venous thrombosis was not known until recently. In 1993, Dahlback *et. al.,* for the first time uncovered that the APCR to be almost exclusively inherited and possibly by autosomal dominant trait. In 1994, Bertina *et. al.,* discovered the cause of APCR to be a mutation in the Factor V gene, a purine transistion G to A at nucleotide position 1691, resulting in mutant Factor V arg506gln called Factor V Leiden (FVL). This R506Q change in the Factor V protein confers resistance to the proteolytic action of APC resulting in unregulated clotting process and makes carriers and homozygotes of FVL lifelong susceptible to risk of 10-fold in heterozygotes and 80-fold in homozygotes to venous thrombosis. Over 90% of familial and over 20% of sporadic thrombophilia are caused by FVL.

Earlier population studies revealed a wide range in the carrier frequency of FVL, from 15% in Greeks to 5-10% in other Europeans, and 0% in Africans and Asians. However, recent American literature revealed about 5% incidence of FVL in white-Americans and 1-3% in African-Americans. The disparity in the frequencies was probably due to the smaller sample size selected or due to the geographic differences. The frequency of FVL in Louisiana is unknown. Thus, the goal of the present investigation was to establish the prevalence of FVL in Louisiana using a larger population study so that meaningful frequency data were obtained in the two races. Data and statistics, methodology used and the break down of gene frequency in different races of Louisiana will be presented.

Program Nr: 2215 from the 1999 ASHG Annual Meeting

Comparing Linkage Disequilibrium Strength Within and Between Population and Genomic Regions. *S.K. Nath[1], M. Blumenfeld[2], I. Chumakov[2], L. Bougeleret[2], A. Cohen[2], L. Essioux[2], D. Cohen[2], N.J. Schork[1,2,3,4].* 1) Epidemiology and Biostatistics, CWRU, Cleveland, OH; 2) Genset, Paris, France; 3) Porgram for Population Gentics, Harvard University, Boston, MA; 4) The Jackson Laboratory, Bar Harbor, ME.

With the emergence of high-density genetic maps of the human genome (as well as other species genomes) has come the need to assess the behavior of alleles at the polymorphic sites constituting the maps. Linkage disequilibrium (LD) is one important population genetic phenomenon that needs to be assessed empirically if the utility of genetic maps for population-based studies is to be put into perspective. We have developed a number of methodologies and study designs for testing and comparing the strength of LD among a set of linked markers within and across different populations and different genomic regions. We showcase the use of these methods by applying them to single nucleotide polymorphism data collected on a large number of individuals at a number of chromosomal regions. We discuss the implications of our results as well as items that need to be considered in any study attempting to qualify LD behavior.

Program Nr: 2216 from the 1999 ASHG Annual Meeting

Association Study of the B3-adrenergic receptor polymorphism with risk factors for colon cancer. *D.H. Nguyen[1], R.C. Bada[1], C.L. Bird[1], R.W. Haile[1], H.D. Frankl[2], E.R. Lee[3], S.A. Ingles[1]*. 1) Preventive Medicine, USC/Norris Cancer Center, Los Angeles, CA; 2) Division of Gastroenterology, Sunset Kaiser Permanente Medical Center, Los Angeles, CA; 3) Division of Gastroenterology, Bellflower Kaiser Permanente Medical Center, Los Angeles, CA.

The B3-adrenergic receptor (B3-ADR) is expressed in visceral adipose tissue in humans, which functions as a lipid oxidizer, regulator of thermogenesis, and regulator of resting metabolic rate. Studies have identified the B3-ADR polymorphism as a factor in obesity in different ethnic cohorts. Our hypothesis is that carriers of the B3-Andrenergic Receptor polymorphism may have an increased BMI (body-mass index), insulin levels, triglyceride levels, and an increased likelihood for polyp development (a precursor to colon cancer). The subjects include a case group of 562 subjects with polyps, and a control of 529 subjects without polyps. All subjects were 50+ years of age and underwent sigmoidoscopies. 52% were Caucasians, 17% African American, 18% Hispanic, and 12% Asian. Current results, from BstNI enzyme genotyping of 353 subjects indicate that 18% of the subjects carry at least one variant allele. Carriers had significantly higher levels of triglycerides (median = 169) compared to non-carriers (median = 140, P = 0.01). C-peptide levels were also higher among males who carry a variant allele (median = 2.9) compared to male non-carriers (median = 2.4, P = 0.02). C-peptide levels did not differ by genotype among females. Possible explanations for these results will be discussed.

Program Nr: 2217 from the 1999 ASHG Annual Meeting

The power and robustness of microsatellite-based tests of population expansion. *G.D.C. Nicholson.* Department of Statistics, University of Oxford, Oxford, U.K.

A number of recent investigations have used hypothesis tests based on variation at microsatellite loci to infer human demographic histories. In any such investigation, robustness of the relevant tests to deviations from the genetic assumptions must be ascertained. Furthermore, knowledge of the power of the tests is extremely useful, not only at the planning stage, but also when interpretating the results. The tests described in this study are based on the following statistics: the *F*-statistic, introduced by Di Rienzo *et al.* (Genetics, 148, 1998), the imbalance index, introduced by Kimmel *et al.* (Genetics, 148, 1998), and the *k*- and *g*-statistics, introduced by Reich and Goldstein (PNAS, 95, 1998). Under the stepwise mutation model, *g* is the reciprocal of *F*; hence the tests based on these two statistics have identical properties. With the intention of providing insights into the power and robustness of each of the three distinct tests, coalescent simulations are conducted for a wide range of demographic, genetic and sampling parameter values. The results of the analysis may be grouped into four sections: (i) A broad comparison of the power of each of the tests under the stepwise mutation model and under the assumption of a constant mutation rate across loci (stepwise growth is also assumed). (ii) An assessment of the robustness of the tests to the introduction of non-stepwise mutation models. (iii) An assessment of the effects of introducing mutation rate variability across loci. (iv) A description of two methods for estimating the power of the *F*-test (i.e. without resorting to simulation). The first method is based on the observation that, in a certain limiting region of parameter space, the power may be be expressed as a function of two parameters: the number of loci sampled and the time since expansion (in units of the initial population size). The second method is based on *G* (defined as the ratio of the expected values of the numerator and denominator of *F* under the stepwise growth scenario). Large values of *G* correspond to large values of the power of the *F*-test. Critical values of *G* are calculated such that if $G > G_{crit}$, then the power > 0.90.

Program Nr: 2218 from the 1999 ASHG Annual Meeting

The probability of identity by descent for unrelated similar haplotypes. *I.M. Nolte, G.J. te Meerman.* Medical Genetics, University of Groningen, the Netherlands.

In our department a new method is developed for mapping genes contributing to complex diseases. This method is based on the length of a genomic region that pairs of haplotypes share. The idea behind it is that patients do not only share more often a disease allele but moreover that they share longer segments surrounding the disease allele. This latter hypothesis arises from the fact that it is likely that patients have a more recent common ancestor for the alleles on the disease locus than controls. Therefore less recombination events have shortened the region shared on either side of the disease locus. We believe that this method is suitable for fine-mapping genes to a region of less than 50 kb. By comparing haplotypes that are with appreciable probability identical by descent we try to detect a small haplotype that is present in most of the patients and that is absent in most of the controls. In order to show that this kind of mapping is possible we demonstrate that on the basis of evolutionary principles statistical support for an interval of 10 kb or less is possible if sufficient marker data are available. We derived a formula for the probability of identity by descent of unrelated similar haplotypes extending over multiple markers. Random mating is assumed and the population is subject to selection, mutation and recombination. We also assume that the population has grown from generation to generation using a discrete generation model. With the formula derived the effects of the population size at the last generation, the number of similar haplotypes in the sample, the sample fraction, the recombination fraction, the mutation rate, the selection coefficient, the growth rate of the population of haplotypes and the allele frequencies per locus on the IBD-probability are studied.

Program Nr: 2219 from the 1999 ASHG Annual Meeting

The spectrum of pathogenic mutations and mtDNA haplogroups among Danish lineages manifesting Leber hereditary optic neuropathy. *S. Nørby[1], J. Saillard[1], P. Magalhães[2], M. Schwartz[2], T. Rosenberg[3]*. 1) Institute of Forensic Medicine, University of Copenhagen, Denmark Copenhagen, Denmark; 2) Laboratory of Molecular Genetics, Rigshospitalet, Copenhagen, Denmark; 3) National Eye Clinic for the Visually Impaired, Hellerup, Denmark.

Twenty-nine Danish lineages manifesting Leber hereditary optic neuropathy (LHON) have been analyzed for their pathogenic mitochondrial DNA (mtDNA) mutation as well as sequenced in both hypervariable segments, HVS I and II, of the mtDNA control region. Twenty-five lineages (86%) were found to have the ND4/11778 transition, two (7%) the ND1/3460 transition, and one (3.5%) the ND6/14484 transition. In one lineage the pathogenic mutation has not been identified. Based on HVS I sequences, and information about position 73 in HVS II, twenty different haplotypes were identified which could be allocated to haplogroups H, U, K, T, and J in a phylogenetic network. In contrast to most other studies on LHON in Caucasians we found the same low frequency (12%) of haplogroup J among Danish 11778 LHON lineages as in our controls. In twenty-three of the twenty-five11778 lineages we obtained information about the polymorphism at position 11719: twelve lineages, belonging tohaplogroups U, K, T and J, had the variant 11719A, whereas the remaining eleven, all haplogroup H, had the 11719G of the reference sequence indicating that A is the ancestral nucleotide.

Program Nr: 2220 from the 1999 ASHG Annual Meeting

Use of disease heterogeneity to search for genes of a multifactorial disease, rheumatoid arthritis. *M. Pascanu*[1, 2]. 1) for ECRAF, the European Consortium on Rheumatoid Arthritis Families; 2) Pole Genetique / LREPA, Universite Paris 7, Paris, France.

Rheumatoid arthritis (RA), the most frequent autoimmune disease with a prevalence of 1% in adults, is clinically and genetically heterogeneous. About 4/5 of patients develop an agressive disease with joint erosions on X-ray examination. HLA-DRB1 alleles associated with the disease are present in 3/4 patients. A genome scan in 90 caucasian RA sib-pair (ASP) families revealed 26 candidate susceptibility loci (linkage $p<0.05$) outside of the HLA locus. The aim of this paper was to take into account the disease heterogeneity in the analysis.

Affected sib-pair analysis was performed on the publicly available genome scan data (www.genethon.fr) with the ANALYZE package in sub-groups of families defined by 1) disease with joint erosions (n=61) and 2) concordance at the HLA locus : HLA-concordant (n=32)(RA sibs with identical genotypes) or -discordant ASP (other families). New candidate loci were defined by markers with linkage evidence $p<0.05$.

Erosive RA genome scan analysis showed 18 candidate loci (4 new). We observed 22 loci (7 new) in HLA-concordant ASP and 10 loci (2 new) in HLA-discordant ASP. Interestingly, they were significantly more loci observed in HLA conc. than disc. ASP ($p<0.05$), suggesting that HLA factors interact with other susceptibility genes. Out of the 12 new candidate loci, one was common to the erosive and HLA conc. groups, suggesting a gene interacting with HLA factors in predisposing to agressive disease. Out of the 26 known candidate loci, 5 more such loci showed similar suggestion and 6 loci were found both for erosive and HLA-disc. groups, suggesting genes involved in agressive disease independently from HLA factors.

In conclusion, taking into account clinical and genetic heterogeneity results in the identification of new candidate loci and provides clues for the search of the underlying susceptibility genes.

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Program Nr: 2221 from the 1999 ASHG Annual Meeting

Extraction of DNA from Buccal Swabs Using Various Protocols. *H.M. Patterson, S. Knapp, C.B. Sutcliffe, J.L. Haines.* Program in Human Genetics, Department of Molecular Physiology and Biophysics, Vanderbilt University Medical Center, Nashville, TN.

Buccal swabs are useful as a less invasive alternative to blood collection for obtaining cells for DNA extraction. Therefore, it is of critical importance to both diagnostic and research laboratories that a technique for DNA extraction from buccal swabs be developed that is robust and reliable in the hands of multiple users. The goals of this project were to determine the optimal buccal swab extraction kit protocol based on the stability of the DNA over the course of one year and our ability to extract DNA from buccal swabs stored for over one year.

DNA extraction kits from various manufacturers were tested along with the laboratory standard boiling procedure. Twenty-five individuals submitted two buccal swabs each, one for immediate extraction with one of the five protocols and the other stored at 4°C for processing one year from the date of the original extraction. Five buccal swabs were extracted with each of the kits using the manufacturers recommended protocol, as well as the standard boiling procedure. Following extraction, the DNA was quantitated using PicoGreen™ (Molecular Probes) and stored at 4°C. The DNA from each individual's buccal swab was amplified and run out on an acrylamide gel once every month with the exception of the samples from the Purgene™ (Gentra) kit and the standard procedure, which did not yield the expected bands at the onset of the experiment and were therefore excluded from further analysis. The first PCR was done less than one month from the date of the original extraction using D4S2377 primers. Subsequent PCRs were carried out using Cy-3 labeled Amelogenin primers. The samples from the Qiagen™, Promega™ and Epicentre™ kits continued to present the expected bands with all consecutive PCRs. By evaluating each gel, it was determined that the Qiagen™ kit produced the most desirable results of the five protocols tested. The PCR on the year-old buccal swabs was performed successfully using this kit in conjunction with the Amelogenin primers.

Program Nr: 2222 from the 1999 ASHG Annual Meeting

Consanguinity rate in Sultanate of Oman. *M.A. Patton[1], A. Rajab[2].* 1) Medical Genetics, St Georges Hospital, London, UK; 2) Genetics Department, Royal Hospital, Muscat, Oman.

The Sultanate of Oman is located in the north east corner of the Arabian peninsula. It has a population of 1.5 million and has now a comprehensive healthcare system. Like many countries in the Middle East there has been a cultural tradition of consanguineous marriages, but the degree of consanguinity had not been delineated. A questionnaire based study was therefore carried out on 60,635 couples attending antenatal clinics. This represented 20% of the population of child bearing age. It was found that 24.1% of marriages were between first cousins and 11.8% between second cousins. A further 20.4% of marriages were between members of the same tribe and because of the strictly endogamous nature of tribal marriages these were also consanguineous to some degree. The coefficient of inbreeding was calculated as 0.0198.

Program Nr: 2223 from the 1999 ASHG Annual Meeting

HSP70-1 promoter polymorphisms in AD sibships-NIMH Genetics Initiative AD Study Group. *R.T. Perry, J.S. Collins, L.E. Harrell, R.C.P. Go.* Epidemiology, Univ Alabama-Birmingham, Birmingham, AL.

As part of the NIMH Genetics Initiative AD Study Group, we recently reported evidence of linkage to 6p21.3 in 266 AD families with two or more affected siblings. One candidate gene that is located in this area is the heat shock protein, HSP70-1. Heat shock proteins are a family of evolutionarily-conserved proteins that help stabilize proteins and their structure, preventing aggregation, especially during periods of cell stress. Aberrrant proteolytic processing of the APP is implicated in AD pathology and the APP promoter contains a stress response element which responds to stress. Hsp70 proteins are also associated with NFTs in AD and the microtubule-associated protein tau co-immunoprecipitates with hsp70. In addition, a novel HSP70 mRNA has been reported to be expressed in patients with major depression, but not in normal subjects. We have genotyped a polymorphism located at +190 (G®C) of the 5 UT region of HSP70-1 in a set of 406 affected and unaffected siblings from 125 families. Non-parametric analyses was performed by the S.A.G.E. SIBPAL program. The allele frequencies were b1=0.64 and b2=0.36 and the genotype frequencies were found to be in Hardy-Weinberg equilibrium. Using SIBPAL, no significant increase in allele sharing was found in the affected siblings (p=0.19). A sibling association test using unaffected siblings as controls, SIBASSOC (Curtis D., 1997), found no significant association with any allele at this locus (single-allele association test, p=0.59). However, we are genotyping an additional 179 siblings and will analyze these data using a sibship disequilibrium test. We are also typing a second polymorphism located in the promoter region and the two polymorphisms will be analyzed as a haplotype to increase their informativeness.

Program Nr: 2224 from the 1999 ASHG Annual Meeting

Segregation analysis of phenotypic subtypes of dyslexia. *D. Peterson[1], E. Wijsman[1], K. Goddard[2], L. Hsu[3], V. Berninger[1], W. Raskind[1]*. 1) Dept. Biostatistics, University of Washington, Seattle, WA; 2) Case Western Reserve University, Cleveland, OH; 3) Fred Hutchinson Cancer Research Center, Seattle, WA.

There is evidence for a genetic contribution to reading disability (RD), or dyslexia. Many phenotypic measures are used to diagnose RD. Most are continuous, and can be grouped into phonological, orthographic, and rapid automatized naming measures. Within each of these there are multiple measures that are used by different researchers. Etiologic and phenotypic heterogeneity are complications in genetic analysis, and it would therefore be useful to determine whether component phenotypes provide evidence for major gene inheritance.

We are studying the genetic basis of 24 widely used phenotypic components of RD in 102 nuclear families ascertained through a proband with RD. Based on earlier family aggregation studies, we identified 5 phenotypes that warranted more extensive modeling of the mode of inheritance: word attack (WA), digit span (DS), WRAT-3 spelling (WS), nonword memory (NM), and pseudoword reading efficiency (PR). For each, we have carried out complex segregation analyses using two approaches. The first is a Monte Carlo Markov chain (MCMC) analysis, which estimates both the number of underlying trait loci (QTL) and their effects. The second is the regressive model approach to segregation analysis. NM and DS provided the strongest evidence for major gene effects; WA provided no evidence for major gene effects; PR and WS provided modest evidence for major gene effects. For NM, the MCMC analysis estimated a posterior probability of >98% as >1 QTL, with an estimate of 1-3 such QTL. For digit span, the posterior probability of >1 QTL was >88%, with an estimate of 1-3 QTL. For NM, complex segregation analysis suggested a Mendelian model with homozygous means separated by 3.2 sds, and a heterozygote .8 sd from the higher mean. Age, sex and verbal IQ were all significant covariates. For DS, the best model was a dominant mode of inheritance with homozygous means separated by 1 sd. Age and verbal IQ were significant covariates. These results pave the way for gene mapping with these phenotypes.

Program Nr: 2225 from the 1999 ASHG Annual Meeting

A new statistical approach to identify non-pathogenic mutations. Analysis of CFTR gene mutations in a random population. *P.F. Pignatti[1], C. Bombieri[1], S. Giorgi[2], S. Carles[3], R. De Cid[4], F. Belpinati[1], C. Tandoi[2], N. Pallares-Ruiz[3], C. Lazaro[4], B.M. Ciminelli[2], M.C. Romey[3], T. Casals[4], F. Pompei[2], G. Gandini[5], M. Claustres[3], X. Estivill[4], G. Modiano[2]*. 1) Section of Biology and Genetics, DMIBG, Univ. Verona, Italy; 2) E.Calef - Dpt. of Biol., Univ. Roma - Tor Vergata, Italy; 3) Inst. Biology, Univ. Montpellier, France; 4) Medical and Mol. Genet. Center, Hospital Duran i Reynals, Barcelona, Spain; 5) Blood Center, Verona Hospital, Italy.

It may be difficult to determine the role of a not obviously pathogenic mutation in causing disease. We here propose a conceptually straightforward method to identify 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 CF mutations: in the CFTR gene more than 800 different mutations have been described. The analysis does not exclude the possibility that, in some individuals, particular combinations of alleles not causing CF may cause attenuated or atypical phenotypes. A total of 191 DNA samples (sufficient to pick up essentially all mutations with a frequency of at least 0.01) from random individuals from Italy, France, and Spain, were investigated by DGGE analysis of all the coding and proximal non coding regions of the gene. A total of 43 mutations was detected. Three mutations (DF508, N1303K, G85E) were known to be CF-causing. Four were new mutations: 1341+28C/T, 2082C/T, L1096R, and I1131V. Ten mutations (125G/C, 875+40A/G, TTGAn, IVS8-6polyT,1525-61A/G, M470V, 2694T/G, 3061-65C/A, 4002A/G, 4521G/A) were classified as non CF-causing alleles on the basis of their frequency. The remaining ones have a cumulative frequency far exceeding q, therefore most of them cannot be CF-causing mutations, but the actual frequency data do not allow their classification. An analysis on a larger sample will clarify their role.

Program Nr: 2226 from the 1999 ASHG Annual Meeting

The San Antonio Family Study of Diabetic Nephropathy in Mexican Americans. . *R. Plaetke[1], P. Pergola[1], S. Eaton[1], R. Duggirala[2], N. Arar[1], C. Diaz[1], M. Stern[2], H. Abboud[1].* 1) Division of Nephrology; 2) Division of Epidemiology; University of Texas Health Science Center at San Antonio, San Antonio, TX.

Diabetic nephropathy (DN) is the most common cause of end-stage renal disease (ESRD). The prevalence of DN is 5-10% in Caucasians with type 2 diabetes (T2DM), but 30-40% in Mexican Americans. In addition, Mexican Americans have a prevalence of T2DM 3 times higher than Caucasians. Studies show that DN can cluster in families and has a genetic component. Little is known about the genetics of DN in Mexican Americans. Therefore, we established a family ascertainment center at the UTHSCSA to enroll families with a clustering of DN in a gene search study using variance components analysis (VCA). To date, we have ascertained 17 pedigrees consisting of ~465 individuals (total of 128 T2DM cases and 39 DN cases) through probands with ESRD secondary to T2DM; 4 additional pedigrees are being identified. On average, each pedigree has 7.5 individuals with T2DM (range: 2-19) and 2.3 cases with DN (range: 1-7). The average age of T2DM onset is 41.2 years (range: 17-61), and of DN onset is 53.8 years (range: 39-64). Phenotyping of family members for T2DM and DN will be established by interviews and urine and blood analyses (fasting serum glucose, serum creatinine, daily protein/albumin urinary excretion). Data from all 21 ascertained families will be presented for (1) ages at onset of T2DM/DN and years elapsed between diagnoses of both diseases, (2) ethnic backgrounds (besides Mexican American), (3) clustering of the two diseases in the pedigrees, and (4) results of the ongoing phenotyping for T2DM and DN. Preliminary power estimates for VCA, using data from 50 extended families with similar pedigree structures to the original 17 families, show that we will have sufficient power to detect DN genes. Research is funded by the San Antonio Area Foundation.

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Program Nr: 2227 from the 1999 ASHG Annual Meeting

Inference on relationships by IBS allele sharing: the mean number of loci per individual needed to discriminate full sibs from non-relatives in a sequential test. *S. Presciuttini[1,2], L. Casarino[3], S. Verdiani[3], A. Mannucci[3], L. Peirano[3], F. De Stefano[3], J.E. Bailey-Wilson[2].* 1) Dept. Uomo e Ambiente, Univ. Pisa (I); 2) NIH/NHGRI, Baltimore, MD; 3) Ist. Medicina Legale, Univ. Genoa (I).

We studied the discrimination between pairs of full sibs and pairs of unrelated individuals based on the total number of alleles identical by state at a number of loci (Y). The analysis was based on a sample of assumed sib pairs, the donors and their sibling recipients of bone marrow transplantation, typed at 13 markers. We obtained the distributions of Y in: 1) these pairs of siblings; 2) a simulation of 10,000 sib pairs using the allele frequencies estimated from the sample; 3) a sample of unrelated individuals generated by selecting one sib per pair. We also chose a marker with heterozygosity intermediate between those used in the real analyses, and computed the exact probabilities of observing a given Y for both sib pairs and non-relative pairs, for each number of markers (M) from 2 to 50. The accordance between the observed distribution of Y among sib pairs and that expected on the basis of the simulation was satisfactory. The accordance between the distribution among both non-relatives and simulated sib pairs and the exact distributions was also excellent. We then explored the discrimination power of a sequential test based on a first set of ten markers and the addition of five further markers at each step, after removing the pairs that were already discriminated. Two null hypotheses were considered: i) that a given pair was assumed to be non-relatives (and we wanted to obtain the probability that these two subjects were full sibs), and ii) that a given pair was assumed to be full sibs (and we wanted to obtain the probability that these individuals were unrelated). The discrimination of full sibs from unrelated individuals with $P<0.001$ was reached with an average number of 16.4 markers typed per individuals, at a power of 0.997. Similarly, the discrimination of unrelated individuals from full sibs was reached with an average of 15.8 markers per individual. The same power would have been reached by a non-sequential test with 40 markers per individual in both cases.

Program Nr: 2228 from the 1999 ASHG Annual Meeting

Y-CHROMOSOMAL DNA VARIATION IN PAKISTAN. *R. Qamar[1,2], Q. Ayub[1,2], A. Mohyuddin[1,2], T. Zerjal[2], S.Q. Mehdi[1], C. Tyler-Smith[2]*. 1) Biomedical & Gen. Engg. Labs, Islamabad, Pakistan; 2) Dept. Biochemistry, University of Oxford, Oxford, UK.

Pakistan lies on the path of the postulated early migration route out of Africa, but little is known about the origins of its current populations. Of particular interest are the Burusho who speak Burushaski, one of the world's remaining language isolates. The Kalash Kafirs are allegedly descendants of soldiers who accompanied Alexander the Great. The Baltis are considered to be of Tibetan origin, while the Hazara are Mongoloid and probably of Tatar descent. Populations in southern Pakistan of interest are the Balochi (Aleppo origin), the Makrani (Negroid) and the Parsis who are recent immigrants from Uzbekistan/Iran.

We are using Y-chromosomal DNA variation to investigate the genetic history and relationships of these populations. More than 750 males from 13 populations have been analysed with 16 biallelic markers: YAP, SRY -8299, sY81, Apt, 12f2, RPS4Y 711, M9, M20, LINE-1, SRY +465, LLY22g/HindIII, Tat, 92R7, SRY -2627, SRY -1532 and M17. These markers identify 16 haplogroups in worldwide populations, 10 of which were found in Pakistan. North-east to south-west geographical gradients of haplogroup distribution are seen, but there is no obvious correlation with language; despite speaking an isolated language, the Burusho Y-chromosomal haplogroup distribution resembles those of surrounding populations. Ten existing and several novel Y microsatellites are also being scored to investigate variation within the haplogroups.

Program Nr: 2229 from the 1999 ASHG Annual Meeting

Polymorphism of fibrinogen genotypes of three ethnic groups in multiracial Singapore. *S.C. Quek[1], C.K Heng[1], S. Hong[1], N. Saha[2], P.S Low[1]*. 1) Department of Pediatrics, National Univ Singapore, Singapore, Singapore; 2) Department of Human genetics, Graduate School of Public Health, University of Pittsburgh.

Plasma fibrinogen is reportedly associated with a higher risk of coronary artery disease (CAD) in epidemiological studies. Racial variation and difference between sexes have also been described. We investigated newborn cord blood levels of fibrinogen in term babies of three ethnic groups (namely, Chinese, Malays and Indians) in Singapore, as well as five polymorphisms (*Hind*III, *Ava*II, *Bcl*I, *Taq*I, *Rsa*I sites) on the fibrinogen gene. No significant difference was detected among fibrinogen levels between the two sexes nor was there any interracial difference. The rare allele frequencies of the various polymorphisms are presented below. The genotype frequencies examined were in accordance with a population at Hardy-Weinberg equilibrium. Using the *Z*-test, significant differences ($P<0.05$) were noted between the allelic frequencies between all three groups for all the sites. Unlike in the adults, there was no statistical difference in cord fibrinogen levels between all the genotypes.

	*Ava*II	TaqI	*Rsa*I	*Hind*III	*BclI*
	G^{+1689} (*n*)	*T2* (*n*)	*R2* (*n*)	T^{-148} (*n*)	*B2* (*n*)
Chinese	0.243 (382)	0.436 (399)	0.421 (341)	0.229 (166)	0.244 (293)
Malays	0.298 (282)	0.333 (290)	0.300 (255)	0.313 (131)	0.307 (210)
Indians	0.116 (202)	0.264 (191)	0.262 (183)	0.128 (133)	0.113 (181)

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Program Nr: 2230 from the 1999 ASHG Annual Meeting

A general approach to family based association tests. *D. Rabinowitz.* FaDepartment of Statistics, Columbia University, New York, NY.

A general approach to family based association tests is presented. The approach involves comparing test statistics to their conditional distribution given the minimal sufficient statistics under the null hypothesis for admixture, ascertainment and the genetic model. The approach results in valid type I error rates, therefore, regardless of admixture, ascertainment and the genetic model. There is no restriction on the test statistics, so the approach may be applied with quantitative traits, traits with variable age-at-onset, and dichotomous traits; it may be applied with multiple tightly linked loci and with multiple unlinked loci, and with bi-allelic or multi-allelic loci; environmental and genetic covariates and a substantive knowledge may be incorporated into analyses; and information from multi-generational pedigrees may be combined. When examining association in order to detect linkage, the minimal sufficient statistics are the observed traits in pedigree members and the genotypes of the pedigree founders; when testing for association in the presence of linkage, the minimal sufficient statistics also encompass identity-by-descent relationships; when examining multiple closely linked markers, the minimal sufficient statistics also encompass phase. If pedigree founder genotypes, identity-by-descent relationships or phase are not available, the minimal sufficient statistic is obtained by treating the missing data as the parameter and computing the corresponding minimal sufficient statistic. The approach may therefore be applied with incomplete marker genotypes and arbitrary pedigree structures. Score statistics from regression models for the influence of genotypes and covariates on traits are natural choices of test statistics. Normalization by the conditional expectations and variances given the minimal sufficient statistics results in likelihood based statistics from the conditional likelihood. P-values may therefore be computed by comparing the normalized statistics to central chi-square distributions. Normalization is efficiently implemented by table-lookup of conditional means and covariances of genotype indicators. Joint work with Qiong Yang, Nan Laird, Steve Horvath and Xin Xu.

Program Nr: 2231 from the 1999 ASHG Annual Meeting

Is there an insulin resistance locus on chromosome 1: Evidence from Mexican American hypertension families with direct assessment of insulin sensitivity. *L.J. Raffel[1], R.C. Davis[2], J.I. Rotter[1], W.A. Hsueh[2], A. Xiang[3], S.P. Azen[3], H.N. Hodis[3], J.-Y. Lu[1], J. Diaz[3], E. Toscano[3], L. Castellani[2], K. Krass[2], P.-Z. Wen[2], B. Juarez[2], M. Quinones[2], S. Tan[3], T.A. Buchanan[3]*. 1) Cedars- Sinai Medical Ctr; 2) Univ of California; 3) Univ of Southern California, Los Angeles, CA.

In an ongoing genetic study of Mexican American families ascertained via a proband with hypertension, a 10 cM genome scan is underway testing for chromosomal regions which demonstrate linkage to quantitative traits associated with hypertension, atherosclerosis and insulin resistance. To date, 168 markers have been genotyped (44% of the genome scan) and analyzed using SIBPAL in 390 individuals from 77 families. An important feature of this study is direct measurement of insulin sensitivity by steady state glucose infusion rate (GINF) during a hyperinsulinemic euglycemic clamp. Linkage analysis suggests that there may be a locus important in insulin resistance on chromosome 1. As shown in the Table, there is evidence for linkage of D1S2141 and D1S549 to fasting and 2-hour OGTT insulin and GINF. Of interest, GINF gives the best evidence for linkage, suggesting that direct trait assessment provides more analytic power. It also appears that insulin resistance at this locus contributes to the broader metabolic syndrome, as indicated by the fasting triglycerides data.

Marker	cM	Fast. Insulin	2-Hr. Insulin	GINF	Triglycerides
D1S2141	233		.01	.01	
D1S549	240	.03	.03	.0003	.02

Although a genome scan in the Pima found evidence linking fasting insulin and glucose to chromosome 1q, our region is separate and more distal. Further investigation of this region, including multipoint analysis, is underway to better localize the region of maximal linkage.

Program Nr: 2232 from the 1999 ASHG Annual Meeting

High incidence of Propionyl-CoA-Carboxylase in Greenland due to a founder mutation, 1540insCCC, in PCCB.

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Propionyl-CoA carboxylase (PCC) is a mitochondrial, biotin-dependent enzyme involved in the catabolism of branched chain amino acids, odd chain fatty acids, and other metabolites. PCC consists of two subunits, alpha and beta, encoded by the PCCA and PCCB genes, respectively. Inherited deficiency of PCC due to mutations in either gene results in propionic acidemia (PA), a severe autosomal recessive disease. The disease is clinically heterogenous, often presenting as a lethal neonatal form. Milder forms with a later onset have also been identified. The disease is considered to be very rare with an incidence < 100.000. Due to an observed apparently high frequency of this disorder in the Greenlandic population, we have analyzed RT-PCR products of the beta-subunit mRNA obtained from cultured fibroblasts from Greenlandic patients, in order to characterized the mutation(s) responsible for PA. SSCP analysis followed by sequencing identified a novel mutation,1540insCCC. This mutation was found in homozygous form in three patients and in heterozygous form in one patient. Due to the observed high frequency of PA in Greenland, we have screened 310 anonymous individual for 1540insCCC. We found a carrier frequency of 6per cent, which is much higher than in any other population studied so far. Haplotype analysis using a closely linked marker suggested a common founder of 1540insCCC. Thes observation will have implications for genetic couselling of the involved families.

Program Nr: 2233 from the 1999 ASHG Annual Meeting

Cladistic Diplotype: A novel method for organizing genotype data for statistical analysis. *H. Razzaghi, C.E. Aston, M.I. Kamboh.* Dept Human Genetics, Univ Pittsburgh, Pittsburgh, PA.

Haplotype-based cladistic analysis is a powerful tool to assess genotype-phenotype association. However, several factors limit the wide use of haplotype cladistic analysis in population-based samples. Primary among these is the difficulty in determining multi-locus haplotypes. Subjects with genotypes of indeterminate phase provide no haplotype information and are consequently excluded from the analysis. This limitation becomes increasingly significant as more loci are incorporated in the cladogram. Another limitation is ambiguity in the construction of the cladogram. The structure of a cladogram can vary depending on the perceived evolutionary history among the constructed haplotypes, which can be connected to each other in many ways. We have developed a similar, yet novel algorithm to overcome these limitations. We denote an individual's genotype at multiple variable sites regardless of haplotype assignment as a "diplotype". Genotype data are unambiguously organized into diplotypes by simple cross-classification. Two simple criteria are used to construct a cladogram from diplotypes: gradient distribution and sequential relationship among the diplotypes. Attaching diplotypic phenotype means to the resultant cladogram provides a flow diagram which shows the effect on phenotypic variation of simple mutational changes against a known genetic background. In comparison, univariate (single variable site) multiple regression analysis shows the effect of a simple mutational change against an averaged background which may confound the effects of the mutation amidst, for example, gene interactions. We used this cladistic diplotype approach to examine the effects of six variable sites in the lipoprotein lipase gene on variation in plasma quantitative traits related to homeostasis of lipids, glucose, and insulin. We found several significant associations using cladistic diplotypes which otherwise were nonsignificant in the univariate multiple regression analyses.

Program Nr: 2234 from the 1999 ASHG Annual Meeting

Panorama of Alu insertion polymorphisms in Indian populations. *P.H Reddy[1], M.K. Das[2], K. Das[2], P.A. Reddy[1], N. Malik[3], M.P. Sachdeva[4], S.S. Mastana[5].* 1) NHGRI, NIH, Bethesda, MD; 2) Anthropometry and Human Genetics Unit, ISI, Calcutta, India; 3) Anthropology Department, Ravi Shankar University, Raipur, India; 4) Anthropology Department, Delhi University, Delhi, India; 5) Human Sciences Department, Loughborough University, Loughborough, UK.

Highly dimorphic Alu family members in well-defined populations provide a useful tool to population geneticists for understanding the population dynamics that have occurred over time. We report here a study of Six Alu insertion loci from 15 populations (1200 samples) representing North, Central and East Indian caste and tribal groups. Overall spectrum of variation in these populations is very interesting at different geographical and cultural levels. Average levels of heterozygosities were found to be relatively high in these populations. The genetic diversity coefficient G_{ST} among this group of populations was observed to be high (0.088). Phylogenetic trees and principal components analysis (PCA) computed from Alu frequencies provide support for socio-cultural and geographical assignment of these populations in Indian population structure. Results are discussed with reference to population origins and human evolution in India.

Program Nr: 2235 from the 1999 ASHG Annual Meeting

Familial aggregation of nephropathy-associated phenotypes in pedigrees ascertained for type 2 diabetes. *S.S. Rich[1], J.H. Warram[2], A.S. Krolewski[2].* 1) Dept Public Health Sci, Wake Forest Univ Sch Medicine, Winston-Salem, NC; 2) Dept Genetics and Epidemiology, Joslin Diabetes Center, Boston, MA.

Familial aggregation of nephropathy has been demonstrated in both type 1 and type 2 diabetes. Levels of urinary albumin excretion (UAE) define both the diagnosis of diabetic nephropathy and its progression. Obesity and high blood pressure are also risk factors of nephropathy. In 96 pedigrees (2032 subjects), 1269 individuals had urinary albumin creatinine ratio (ACR), a surrogate of urinary albumin excretion. Analysis using the variance components (SOLAR) was used to estimate heritabilities and familial correlations for log(ACR), systolic blood pressure (SBP), diastolic blood pressure (DBP) and maximum ideal body weight (maxIBW).

Analyses revealed that log(ACR) was heritable (h^2=0.26), as was SBP (h^2=0.41), DBP (h^2=0.29) and maxIBW (h^2=0.63). We had previously shown that log(ACR) was genetically correlated with both SBP (rG=0.27); DBP (rG=0.29), with an increase in diabetics (SBP, rG=36; DBP, rG=0.53). In these pedigrees, using a bivariate analysis, strong genetic (rG) and weak environmental (rE) correlations were observed between maxIBW and SBP (rG=0.41, rE=0.02) and between maxIBW and DBP (rG=0.59, rE=0.06). Using only diabetics, heritability estimates remained similar, but genetic correlations were increased for maxIBW and SBP (rG=0.51) and for maxIBW and DBP (rG-0.66).

These results suggest that the primary determinants for nephropathy-associated traits are strongtly influenced by genetic factors. The high genetic correlations suggest that similar genetic factors may be controlling variation for BP and maxIBW, with small contributions to environmental risk factors. The increase in genetic correlation in diabetes suggest that identification of genes contributing to obesity and hypertension may result in the identification of genes for diabetic nephropathy.

Program Nr: 2236 from the 1999 ASHG Annual Meeting

Family structure and parental affectation status influence the mode of inheritance of HLA-linked rheumatoid arthritis (RA). *A.S. Rigby[1], A.J. MacGregor[2].* 1) Sheffield Children's Hosp, Univ Sheffield, Sheffield, England; 2) Twin Research Unit, St Thomas' Hospital, London, England.

Genetic linkage between RA and the HLA region has been shown using parental haplotype sharing in affected sibpairs. Previous studies have not examined whether the inheritance of RA is influenced by the specific structure of the families ascertained. Recent theoretical developments (Thomson, Am J Hum Genet 1995;57:474-486) take into account sampling schemes based on multiplex sib (MS) and multiplex parent sib (MPS) families. Here, we have used published data from multicase RA families to investigate whether the mode of inheritance of HLA-linked RA is influenced by pedigree type or parental affectation status. A literature search identified 191 nonoverlapping RA sibships (166 MS and 25 MPS pedigrees) consisting of 166 sibpairs, 19 sibtrios, 4 sibquads, 1 set of quintuplets and 1 set of sextuplets. Parental HLA haplotypes (A,B,C and DR loci) had been assigned to the RA sibships. In the group as a whole, the RA sibpairs shared 2,1,0 haplotypes in ratio of 0.39:0.43:0.18 which was significantly different from random expectations (0.25:0.5:0.25) ($p<0.01$). This distribution was closer to a recessive ($Chi^2=2.95$, $p=0.09$) than an additive model ($Chi^2=5.65$, $p=0.02$). Subgroup analysis by family type showed differences in terms of mode of inheritance. In MS families there was still an excess of haplotype sharing with an observed distribution of 0.42:0.41:0.17. There was no such difference in MPS families. The MPS families were analyzed further by considering whether the 'share 1' haplotype came from either the affected or unaffected parent. The distribution of share 2, 1 (affected), 1 (unaffected) and 0 haplotypes was 9,10,8,8. Both recessive (expected 8.8,8.8,8.8,8.8) and additive (expected 13.2,11.5,6.0,4.3) models were not rejected. Since most MPS pedigrees consisted of an affected female rather than an affected male parent, the presence of maternal-fetal interactions cannot be ruled out. The results point to heterogeneity in the genetic contribution to RA and indicate that family structure needs to be taken into account in linkage and family-based association studies of disease.

Program Nr: 2237 from the 1999 ASHG Annual Meeting

A Bayesian Hierarchical Model for Allele Frequencies. *K.M Roeder[1], J.R. Lockwood[1], B. Devlin[2].* 1) Department of Statistics, Carnegie Mellon University, Pittsburgh, PA; 2) Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA.

Estimates of allele frequencies are required for most linkage studies. For example, founder genotypes must be inferred for analysis of extended pedigrees; likewise, for affected relative pair methods, ibd-sharing must be inferred from allele distributions. When families are drawn from heterogeneous subpopulations, estimating allele and genotype distributions from the total sample could lead to improper inference of linkage. Building on the work of Lange(1995; Genetica 96:107-117) and Roeder et al. (1998; Biometrika 85:269-287), we propose a Bayesian method to estimate the allele distributions for known subpopulations and multiple loci. Given allele counts for a collection of loci and subpopulations, this Bayesian hierarchical model extends existing approaches by allowing for explicit inclusion of prior information about both allele frequencies and inter-population divergence. We describe a methodology for obtaining informative prior distributions for model parameters based on previous research. Using simulated data, we present an application of the model to highlight its features.

Program Nr: 2238 from the 1999 ASHG Annual Meeting

Host genetic determinants of HIV-1 infection in Italy: an epidemiological survey. *V. Romano-Spica[1], A. Ianni[1], D. Arzani[1], M. Orsini[1], L. Cattarini[1], S. Majore[2].* 1) Institute of Hygiene (Dir. Prof. G.C. Vanini) Catholic Univ. Rome Italy; 2) Institute of Medical Genetics, Univ. of Rome "La Sapienza" Rome Italy.

Inherited factors are involved in determining host response to infectious agents. The interaction between host genetic differences and variant viral strains influences HIV-1 infection and disease progression. The protective effect of mutant alleles at chemokine receptor (CCR) loci has important implications for the outcome of HIV-1 infection among exposed individuals, since these receptors are required cellular ports for viral cell entry and spread. Allelic variants of CCR genes have been described. Hypotheses have also been made for dating haplotype origin after recent strong selective events. A 32-bp deletion (CCR5D32) has been shown to confer partial resistance against HIV-1 infection; a point mutation in the CCR2b receptor gene (CCR264I) confers a prolonged AIDS-free survival to carriers. In the present study we evaluated the frequency of CCR5 and CCR2 wild-type and mutant alleles among a representative sample of the Italian population. Genotype frequency was determined after electrophoresis or RFLP analysis of the amplified products. Observed frequencies for CCR5D32 and CCR264I alleles were 0.0454 and 0.0655, respectively. Genotype distribution was in equilibrium as predicted by the Hardy-Weinberg equation. About 21% of the studied population sample was found to be heterozygous either for CCR2 or CCR5 mutated alleles. The observed allelic frequencies were in accordance with estimates reported for Caucasian populations. These results confirmed that CCR5D32 and CCR264I represent two distinct mutations occurred at different times. The dynamic interaction between host genetic determinants, viral heterogeneity and exposure risk factors has been evaluated, analyzing epidemiological data from different countries. The distribution of allelic variants within a population can be considered a measure of genetic susceptibility to HIV infection and disease progression, and a parameter for risk assessment. Studies on hereditary determinants allow a more comprehensive approach to understand the complex interaction between host and infectious agents within a multifactorial perspective.

Program Nr: 2239 from the 1999 ASHG Annual Meeting

Evaluation of a population-based method to identify affected relative pairs with cancer. *P.A. Romitti, R.M. Squire, M.P. Jones, C.F. Lynch.* Dept PMEH, Univ Iowa, Iowa City, IA.

Lung cancer (LCa) and prostate cancer (PCa) are commonly diagnosed neoplasms in the U.S. Previous studies suggest that both LCa and PCa may have genetic and environmental determinants. One approach to evaluate these determinants is to study families with affected relative pairs. Such studies may be most powerful when families are selected from a population where the underlying incidence of cancer is known. To identify population-based families where cancer affects relative pairs, a two-phase approach was used. First, patients with LCa or PCa were ascertained through the Iowa SEER Cancer Registry, and affected first- or second-degree relatives with the same site cancer were identified through review of the patients medical records. Each patient was classified as family history positive (FHP)entry of a relative with the same site cancer, negative (FHN)entry of no relatives with the same site cancer or unknown (FHU)no entry of family history status. Second, all FHP patients were contacted by mail and asked to complete a comprehensive self-administered questionnaire. A random sample of FHN and FHU patients was also contacted to determine the proportion with an affected relative potentially missed by using medical records. Beginning in 1997, abstraction of family history data has been conducted for Iowans diagnosed with cancer. To date, 1006 living LCa (FHP=87; FHN/U=919) and 1482 living PCa (FHP=136; FHN/U=1346) patients have been ascertained. Of these, 150 LCa (FHP=87; FHN/U=63) and 220 PCa (FHP=136; FHN/U=84) were contacted, and presently, 64 LCa (FHP=36; FHN/U=28) and 123 PCa (FHP=86; FHN/U=37) have returned questionnaires. Percentage of concordance for family history status between medical record data and patient self-report tended to be high for both LCa (FHP=92%; FHN/U=79%) and PCa (FHP=85%; FHN/U=87%). These results provide preliminary evidence that collection of family history data during routine cancer surveillance may provide a cost-efficient method to identify affected relative pairs. Future analyses will include evaluation of additional cases and cancer sites and examination of concordance by age and gender of patients and type of practitioner.

Program Nr: 2240 from the 1999 ASHG Annual Meeting

Statistical power and error of different pedigree structures for the detection of genetic linkage in quantitative traits. *M. Ronderos, LD. Atwood.* Division of Epidemiology, University of Minnesota, Minneapolis, MN.

The statistical efficiency of different pedigree structures for the detection of human genetic linkage is not fully understood. This information is important for the design of genetic epidemiology studies. The purpose of this simulation study was to compare the statistical power and error provided by different pedigree structures when the total number of subjects in the study is kept constant. The pedigree structures tested included CEPH families (CF; 2 sibs, 2 parents, 4 grandparents), CEPH families with two grandparents missing (CFM), nuclear families (NF; 2 sibs, 2 parents), nuclear families missing one parent (NFM), and sibships (no parents) of two (S2), three (S3) or four sibs (S4). For each of these groups, a total of 600 data sets were generated. Each data set contained 480 subjects, and the number of pedigrees per data set was adjusted according to pedigree size. The generating model assumed a quantitative trait on which a single gene accounted for 50% of the population variance. The remaining 50% of the variance was explained by random variation. The results indicate that sibships of three or four sibs provide significantly higher statistical power than CEPH families, nuclear families or sib-pairs (P<0.0001). The mean (95% CI) LOD scores for linked markers were 1.58 (1.51, 1.66), 1.39 (1.32, 1.46), 1.17 (1.11, 1.23) and 0.90 (0.85, 0.94) for the CF, CFM, NF, and NFM groups, respectively. The mean LOD scores for S2 were 0.89 (0.85, 0.94). Data sets composed of sibships of three or four sibs presented mean LOD scores of 2.08 (2.01, 2.16) and 3.30 (3.19, 3.49), respectively. The differences between groups were consistent for linked markers located at recombination fractions of 0.01 or 0.05, and for additive or dominant traits. The results were also consistent when the population gene frequency was 0.1, 0.3 or 0.5. The error was significantly higher for CEPH families as compared to nuclear families or sibships (P<0.0001). This study suggests that, given a limited number of subjects, the recruitment of large sibships should be preferred over recruiting either nuclear or CEPH families.

Program Nr: 2241 from the 1999 ASHG Annual Meeting

Paraoxonase (PON1) as a predictor of plasma lipid and lipoprotein levels. *L.S. Rozek, T.S. Hatsukami, R.J. Richter, V.H. Brophy, N.T. Tsai, C.E. Furlong, G.P. Jarvik.* Dept of Medicine, University of Washington, Seattle.

Studies suggest that two common paraoxonase (PON1) polymorphisms, Gln (Q) to Arg (R) at codon 192 ($PON1_{192}$) and Leu (L) to Met (M) at codon 55 ($PON1_{55}$), are associated with cardiovascular disease (CVD). We evaluated 105 Caucasian CVD patients and 153 controls for paraoxonase and diazoxonase activity (POase, DZOase) and $PON1_{192}$ and $PON1_{55}$ genotypes and tested for their correlation with standard lipid and lipoprotein phenotypes. In the entire sample, POase and DZOase showed a small significant positive correlation with apolipoprotein AI (apoAI) (correlations= 0.16, 0.27), high density lipoproteins (HDL) (0.15, 0.19), HDL subfraction 3 (HDL3) (0.18, 0.26), and total cholesterol (TC) (0.13, 0.23), DZOase only with low density lipoprotein cholesterol (LDL-C) (0.17) and apoB (0.12) but not with HDL2, triglycerides (TG) or very low density lipoprotein cholesterol (VLDL-C). $PON1_{192}$ genotype predicted significant variation in HDL3 level (p=0.024) and marginal variation in HDL level (p=0.067) by ANOVA. In cases, POase and DZOase showed a small but significant positive correlation with TC (0.30, 0.30), VLDL-C (0.26, 0.22) and TG (0.23, 0.22); HDL3 and apoAI were significantly correlated with only DZOase (0.23, 0.26). $PON1_{192}$ genotype predicted significant variation in HDL (p=0.006), HDL2 (p=0.019), HDL3 (p=0.009) and marginal variation in VLDL-C (p=0.062) and TG (p=0.059). In controls, POase and DZOase showed a small significant positive correlation with HDL (0.18, 0.20) and HDL3 (0.20, 0.26), DZOase was significantly correlated with apoAI (0.27) and TC (0.18). $PON1_{192}$ genotype did not predict significant variation in any of the measures in the controls. $PON1_{55}$ genotype predicted marginally significant variation in apoB level (p=0.062) only in cases and controls combined. Variation in these traits predicted by any PON1 measure ranged from only 0.1% to 7.4% and appear to differ in cases and controls. DZOase, often not measured in studies of CVD risk, is a better predictor of these traits than PON1 genotype. The association of PON1 and CVD is not expected to be wholly attributable to its small lipoprotein effects. PON1 may be an independent predictor of CVD.

Program Nr: 2242 from the 1999 ASHG Annual Meeting

Cosegregation of single nucleotide polymorphisms in exon I of keratin 14 determined by allele specific sequencing. *E.L. Rugg[1,2], N.J. Wilson[2], G. Magee[2], E.B. Lane[2].* 1) Centre for Cutaneous Research, St Bartholomew's & the Royal London Hospital, Queen Mary & Westfield College London, UK; 2) CRC Cell Structure Research Group, Department of Anatomy & Physiology, University of Dundee, Dundee, UK.

Single nucleotide polymorphisms (SNPs) are the most common type of genetic variation. They are estimated to occur at a frequency of approximately one per thousand base pairs and as such are potentially powerful tools for mapping human genetic diseases. Recent studies however indicate that SNPs do not necessarily occur in an entirely random fashion and that they may show a high degree of disequilibrium making them potentially useful markers for mapping human migration and evolution.

Exon I of keratin 14 (K14) contains six neutral SNPs within a 525 base pair fragment (Sorensen *et al*, 1999). In the course of establishing a data base of the normal DNA sequence for this region of the K14 gene we identified a novel G/C polymorphism in intron I. Our data suggested the SNPs were linked and that there were only two haplotypes in the population under study. PCR was used to specifically amplify a fragment of DNA from fifty unrelated individuals of British origin which contained all seven SNPs. The products were sequenced using the two PCR primers and a primer specific for the G variant of the intronic SNP. The intronic primer only bound to DNA to which it was an exact match and this enabled us to carry out allele specific sequencing. The data confirmed the SNPs cosegregated into two haplotypes, K14a and K14b. These were present in the samples analysed at a frequency of 0.628 and 0.372 respectively with a genotype frequency of K14a/a, 33.3%; K14a/b, 60.0%; K14b/b, 7.7%. The same two haplotypes were also present in two unrelated individuals of Jewish Askenazi extraction however a third haplotype, which differed from K14b by one base pair, was found in DNA from a Japanese individual. These data suggest that the polymorphisms arose in separate populations and may therefore be useful for studies of population genetics and mapping human migration.

Program Nr: 2243 from the 1999 ASHG Annual Meeting

The contribution of mutations 167delT and 35delG in connexin 26 gene to nonsyndromic deafness in Ashkenazi Jews. *M. Sagi[1], I. Lerer[1], E. Malmud[1], H. Levi.[2], A. Raas-Rothschild[1], D. Abeliovich[1].* 1) Dept Human Genetics, and; 2) Speech and Hearing Center, Hadassah Hebrew University Hospital, Jerusalem, Israel.

Mutations in the connexin 26 gene (Cx26) are associated with nonsyndromic recessive deafness DFNB1. In several Mediterranean populations a single mutation in the Cx26 gene, the 35delG, is the most common mutation for sensoneural deafness. Recently, the 167delT mutation in the Cx26 gene was identified as an Ashkenazi founder mutation with an estimated carrier rate of 4%. In the present study we evaluate the contribution of the 167delT and 35delG mutations to nonsyndromic deafness (NSD) in the Ashkenazi Jews.

Twenty unrelated Ashkenazi or half Ashkenazi Jewish patients with prelingual NSD were tested. In 13 patients biallelic Cx26 mutations were identified and 3 patients were heterozygotes with no other mutation identified on the other allele. Thus, in 79% of a total of 38 Cx26 alleles of Ashkenazi origin, a mutation was detected. The mutations frequencies were: 53% for 167delT, 18% for 35delG, others (50del12, E114G and V27I) were 8%. The haplotypes of both 167delT and 35delG mutations indicate that these are founder mutations. In a control group of 210 unselected Ashkenazi individuals, 13 were carriers of the mutation 167delT, 1 was homozygote for the mutation 167delT and 1 was compound heterozygote 167delT/35delG, giving a carrier frequency of 7.3% for the 167delT mutation and 1.1% for the 35delG.

The expected rate of 167delT homozygous in the Ashkenazi Jews, based on a carrier frequency of 5% (a mean carrier frequency based on our results and Morell et al. 1998), is 1/1600. Based on our results, about 50% of NSD among Ashkenazi is due to the 167delT mutation, therefore the expected rate of all NSD in this population is about 1/800. The observed rate of all NSD in the Israeli Ashkenazi population is around 1/1200. The discrepancy between the expected, as predicted by the carrier frequency, and the observed rate of all NSD in the Ashkenazi may suggest incomplete ascertainment of homozygous 167delT children due to incomplete penetrance or variable expression.

Program Nr: 2244 from the 1999 ASHG Annual Meeting

Genetic polymorphisms in Transforming Growth Factor-b**1 and the risk of coronary heart disease.** *N. Saha, E.K. Luedecking, M.I. Kamboh.* Department of Human Genetics, Univ Pittsburgh, Pittsburgh, PA.

Transforming Growth Factor-b1 (TGF-b1) is a multifunctional cytokine thought to be involved in the pathological development of coronary heart disease (CHD). The aim of this study was to investigate the role of three polymorphisms in TGF-b1 in affecting the risk of CHD. Two of the polymorphisms are located in the promoter region at positions -800 (G®A) and -509 (C®T), and the third in the coding region at codon 263 (Thr®Ile). We screened 201 Chinese patients and 201 controls, and 130 Asian Indian patients and 200 controls using PCR based assays. Although there was no difference in genotype or allele distribution at the -800 polymorphic site between patients and controls in either the Indian or Chinese cohorts, there was significant inter-racial variablity in allele frequency between the two populations ($p<0.0001$). The genotype distribution at the -509 polymorphism was significantly different between patients and controls in both the Chinese ($p=0.016$) and Indian ($p=0.027$) samples. There was significant difference in allelic distribution for the -509 polymorphism between the two populations ($p<0.0001$). There was no statistically significant difference in genotype or allele frequency for the codon 263 polymorphism between patients and controls in either the Chinese or Indian populations. Our data indicates that the -509 polymorphim may affect the risk of CHD in the general population.

Program Nr: 2245 from the 1999 ASHG Annual Meeting

Apolipoprotein E promoter polymorphisms and Alzheimer disease in black populations. *A. Sahota[1], M. Yang[1], S. Gao[2], C. Emsley[2], O. Baiyewu[3], A. Ogunniyi[3], K.S. Hall[2], H.C. Hendrie[2].* 1) Dept. Genetics, Rutgers Univ., Piscataway, NJ; 2) Dept. Psychiatry, Indiana Univ. Sch. Med., Indianapolis, IN; 3) Dept. Medicine, Univ. Ibadan, Ibadan, Nigeria.

We have previously shown that the e4 allele of apolipoprotein E (APOE) is not associated with late-onset probable Alzheimer disease (AD) in Nigerian blacks, but may be a weak risk factor in African-Americans who are e4 homozygotes. The e4 allele frequencies in the two groups were comparable, and were higher than those in Caucasian and Japanese populations, where APOE e4 is strongly associated with AD. However, even in at-risk populations, as many as 50% of e4 homozygotes who survive until age 80 years do not develop AD. Recent studies have suggested that sequence variations in the APOE regulatory regions may increase the risk for AD independently or in combination with the e4 allele. These variations include the 491A/T, -427T/C, -219G/T, and +113G/C polymorphisms (positions are relative to the APOE transcriptional start site) and a T/C mutation at codon 28. In particular, homozygosity for the 491A allele was reported to be associated with increased risk for AD in Caucasian subjects independent of their APOE e4 status. For this study, we examined the relationship between the 491A/T polymorphism and probable AD, with and without the confounding effects of APOE e4, in Nigerian blacks from Ibadan (AD = 25, controls =116) and African-Americans from Indianapolis (AD = 39, controls = 39). These individuals are participating in our on-going community-based study of dementia and AD in elderly black populations (age 65 and over). The A and T allele frequencies for patients and controls within each group, and between the two groups, were not significantly different (mean A = 67.1%, mean T = 32.9%), and the APOE e4 allele frequencies were comparable to those previously reported. Logistic regression models did not detect any consistent relationship between the -491A allele and AD in the two groups, indicating that this polymorphism does not represent an independent risk factor for AD in black populations. Analysis of other regulatory region polymorphisms is in progress. Supported by NIH grant AG09956.

Program Nr: 2246 from the 1999 ASHG Annual Meeting

Polymorphism of noncoding regions of mitochondrial DNA in Evenks. *V.B. Salukov, V.P. Puzyrev, S.A. Eletskaya, T.P. Muravyeva.* Population genetics laboratory, Institute of Medical Genetics, Tomsk, Russia.

Restriction polymorphism of D-loop and V region length polymorphism of mitochondrial DNA were investigated in sample of aboriginal Siberian population, namely Evenks living in Chita region (Russia). The sample consisted in total from 93 non-related individuals. No polymorphism was detected by BamHI, AvaII, Kzo9I endonucleases. The frequencies of other polymorphic sites were: 77.42% for KpnI 16129, 57.00% for AspS9I, 45,16% for HaeIII 16517, and 1.08% for EcoRV 16278. No length polymorphism in V region was detected. There were 7 different haplotypes formed by all the polymorphic sites and two of the haplotypes account for 68.48% of the whole sample. Evenks show less variability of mitochondrial DNA comparing with Russians, Mongols, Tuvinians, and Buryats studied earlier with the same set of markers. However, some features of mtDNA polymorphism characterize Evenks as a typical Mongoloid population.

Program Nr: 2247 from the 1999 ASHG Annual Meeting

A two-stage design for linkage disequilibrium. *J.M Satagopan, C.B Begg, E.S Venkatraman.* Epidemiology and Biostatistics, Mem Sloan-Kettering Canc Ctr, New York, NY.

BACKGROUND: We address the optimization of the design of studies of linkage disequilibrium, where one wishes to search for correlation between markers of disequilibrium and disease for a large number of markers. METHOD: We propose a two-stage design when the principal design constraint is the total cost of marker studies, rather than the total number of subjects. It is cost-effective to perform a two-stage design where in the first stage all the markers are evaluated on an initial group of patients, and in the second stage only the most promising markers are further evaluated on all the patients, thus eliminating wastage of resources on markers unlikely to be associated with disease on the basis of the results of the first stage. RESULTS AND CONCLUSION: Using simulation results we show that, as a general guideline, screening all the markers on approximately 25% of the patients and then evaluating the most promising 10% of the markers on the remaining patients provides close to the optimal power for a broad range of parametric configurations.

Program Nr: 2248 from the 1999 ASHG Annual Meeting

Comparison of the power of the transmission distortion test (TDT) for affected and unaffected trios when disease is highly prevalent. *L.J. Scott[1], A. Krolewski[1], J.J. Rogus[2]*. 1) Genetics & Epidemiology, Joslin Diabetes Ctr, Boston, MA; 2) Program for Population Genetics, Harvard School of Public Health, Boston, MA.

TDT was developed to avoid bias due to population stratification in testing candidate genes and works by sampling affected children and their parents (Aff Trios). The use of unaffected trios (Unaff Trios) as a comparison group has been advocated to rule out segregation distortion, but under certain conditions can also be used to effectively detect transmission distortion. To determine when Unaff Trios might have more power than Aff Trios to detect transmission distortion, we calculated the sample sizes necessary both groups for 80% power and 5% type 1 error for a variety of genetic models. Sample sizes were estimated using a Gaussian approximation. The table shows an example of the sample size (N) of Aff Trios relative to Unaff Trios for a dominant model with a risk ratio of 2 and a disease allele frequency of 0.5. The baseline penetrance was varied from 0.2 to 0.4.

Baseline Penet	Disease Prevel	N Aff Trios	N Unaff Trios	Ratio Aff/Unaff
0.2	0.35	479	1711	0.3
0.3	0.53	479	408	1.2
0.4	0.70	479	91	5.2

The sample size of the Unaff Trios decreased with increasing baseline penetrance, whereas the sample size remained constant in Aff Trios. In general, Unaff Trios power exceeds that of the Aff trios when the disease prevalence is greater than 45-55%. In highly prevalent diseases, selection of Unaff Trios with little risk of developing disease in the future may provide a more powerful test than the use of Aff Trios.

Program Nr: 2249 from the 1999 ASHG Annual Meeting

Increased haemochromatosis gene (HFE) mutation in alpha-1 antitrypsin (PiZ) deficient heterozygotes. *M.J. Sharrard[1], A.S. Rigby[1], S. Povey[2], M.S. Tanner[1].* 1) Dept Paediatrics, Univ Sheffield, Sheffield, UK; 2) University College, Univ London, UK.

Alpha-1 antitryspin deficiency (A1ATD) and genetic haemochromatosis (GH) are highly prevalent disorders in the United Kingdom (U.K.) Adults with GH, and both homozygotes and heterozygotes for A1ATD are at increased risk of chronic liver disease, with hepatic deposition of iron. Low penetrance mutations in the haemochromatosis (HFE) gene may interact with the A1ATD heterozygote state to exacerbate liver disease. This study aimed to determine the frequency of HFE gene mutation in those UK individuals carrying the A1ATD mutation. 113 parents (54 mothers, 59 fathers) of PiZZ A1ATD patients, and 1430 healthy controls were genotyped for the C282Y and H63D mutations of the HFE gene using the polymerase chain reaction and restriction enzyme digestion. Data was analysed by relative risk (RR) and 95% confidence intervals (95%CI). In the study group, the H63D allele frequency was significantly elevated (frequency 0.23 and 0.14 for controls, RR 1.67, 95%CI 1.30-2.15), while the C282Y allele frequency was not significantly elevated (0.09 and 0.07 for controls, RR 1.29, 95%CI 0.84-1.98). The frequency of haplotypes associated with the haemochromatosis phenotype was significantly higher in the study group than in controls (12% versus 4%, RR 2.95, 95%CI 1.66-5.21). In the UK, it is probable that racial admixing of populations in which the HFE and A1ATD mutations arose accounts for the high coprevalence detected in this study. There may be selective pressure to maintain this coprevalence as a result of increased fertility. However, increased awareness of HFE gene mutations should lead to investigation of A1ATD liver disease patients for HFE gene mutation, as GH may be a treatable compounding factor.

Program Nr: 2250 from the 1999 ASHG Annual Meeting

Higher than expected carrier rates for familial Mediterranean fever in various Jewish ethnic groups. *M. Shohat, N. Stoffman, T. Shohat, R. Lotan, S. Koman, A. Oron, Y. Danon, G. Halpern, N. Magal.* Department of Medical Genetics and Immunology, FMRC, Schneider Children's Medical Center of Israel, and Rabin Medical Center.Sackler School of Medicine, Tel Aviv University, Israel.

Familial Mediterranean Fever (FMF) is an autosomal recessive disease characterized by recurrent attacks of inflammation of serosal membranes. Amyloidosis leading to renal failure is the most severe complication in untreated patients. In Israel FMF is most frequent among Jews of North African origin. Recently the causative gene (MEFV) has been found and the common mutations characterized. The aim of this study was to investigate the carrier rates of the common MEFV mutations among 400 healthy members of 4 different ethnic groups (100 in each group) in Israel, and to compare the distribution of the different mutations between FMF carriers and patients. We found a high frequency of carriers among Jews from the various ethnic groups. In North African Jews it was 22%, in Iraqi Jews 39%, in Ashkenazi Jews 21%, and in Iranian Jews 6%. The distribution of the 4 most common MEFV mutations among healthy individuals (M694V 29%, V726A 16%, M680I 2% and E148Q 53%) was significantly different ($p < 0.003$) from that found in patients (M694V 84.4%, V726A 9.0%, M680I 0% and E148Q 6.6%). Six healthy asymptomatic individuals were found to carry mutations in both alleles. Of these, 2 were homozygotes for E148Q and 4 were compound heterozygotes, of which 3 were E148Q/M694V, and one was E148Q/V726A. These results demonstrate a very high carrier rate among all Jewish ethnic groups. They confirm that mutation E148Q is associated with a milder phenotype, which explains the lower prevalence of FMF among the Ashkenazi and Iraqi Jews. This study raises the question of the need for molecular screening for M694V homozygotes in the Israeli North African Jewish community.

Program Nr: 2251 from the 1999 ASHG Annual Meeting

Gene characterization using high-risk families: a sensitivity analysis of the MOD score approach. *K.D. Siegmund, W.J. Gauderman, D.C. Thomas.* University of Southern California, Los Angeles.

To estimate penetrance using high-risk families, the MOD score approach maximizes the conditional likelihood of linked marker data given the disease status of all subjects. Several authors have shown that this approach is valid when ascertainment is through the trait only. Easton et al. (1995) apply the method to data from the Breast Cancer Linkage Consortium to estimate the penetrance of BRCA1. In their analysis, the allele frequency, recombination fraction, and baseline rates of disease were fixed and only the estimates of penetrance in carriers were obtained. Because breast cancer is a heterogeneous disease with two known susceptibility genes, they restricted their analysis to families having a LOD score above a given threshold at markers near BRCA1. As they note, such ascertainment is related to the distribution of markers and trait values in a pedigree and can lead to biased penetrance estimates. In a simulation study, we investigate the sensitivity of the MOD score approach to mis-specified (fixed) parameters, unmodeled GxE interaction, and ascertainment based on a LOD score criterion.

We simulate one marker locus linked to a disease locus (q = 0.05). The marker locus has eight alleles and the disease locus two, the frequency of the (dominant) susceptibility allele is 0.01. The penetrance in carriers (p_1) is 0.51 and in non-carriers is 0.05. Results show that carrier penetrance was underestimated when the baseline rate of disease was under-stated (p_1 = 0.36, -29% bias) and overestimated in a sub-sample of families having a LOD ³ 1.0 (p_1 = 0.74, 45% bias). Ignoring a positive GxE interaction resulted in bias only if E was correlated within families (p_1 = 0.66, 29% bias). Maximizing the likelihood over all parameters can eliminate bias of the first and third kind. Bias from the ascertainment on LOD score can be problematic. Easton et al. argue that such ascertainment is not a concern in their data since the majority of information on penetrance comes from families that would have been selected on any threshold. Although this may be true, we caution against the general use of LOD scores for ascertainment.

Program Nr: 2252 from the 1999 ASHG Annual Meeting

Quantitative genetics of blood pressure and adiposity in adults from hypertensive and normotensive families.
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The extent to which blood pressure and body composition share a common genetic basis in individuals with a family history of hypertension vs. individuals from randomly selected families has yet to be fully elucidated. We explored this issue using variance components methods and data from adults in two family studies of body composition and CVD risk - Genetics of Hypertension Study (GHS) families were reqruited through a proband with essential hypertension, whereas Fels Longitudinal Study (FLS) families were randomly ascertained. Using age- and sex-matched data from 450 individuals in 4 large extended GHS families, and 779 individuals in 147 nuclear and extended FLS families, we estimated the heritabilities of systolic and diastolic blood pressure (SBP and DBP), two adiposity measures (body mass index: BMI; and triceps+subscapular+suprailiac skinfolds: SUM3) and two fat pattern indices (subscapular/triceps: STI; and subscapular/subscapular+suprailiac: RFPI), and the additive genetic correlations between SBP and DBP and these adiposity and fat pattern measures. Heritabilities (±SE) from the GHS vs. FLS data were all significant: SBP = 0.38±0.09 vs. 0.27±0.07, DBP = 0.33±0.09 vs. 0.30±0.07, BMI = 0.46±0.10 vs. 0.45±0.07, SUM3 = 0.21±0.11 vs. 0.32±0.09, STI = 0.21±0.07 vs. 0.36±0.11, and RFPI = 0.33±0.10 vs. 0.32±0.08. Genetic correlations (±SE) from best models of the GHS vs. FLS data were: SBP&BMI = 0.77±0.15 vs. 0.71±0.13, SBP&SUM3 = 0.53±0.26 vs. 0.62±0.18, SBP&STI = 0.64±0.24 vs. (0), SBP&RFPI = 0.47±0.26 vs. (0), DBP&BMI = 0.65±0.14 vs. 0.68±0.13, DBP&SUM3 = 0.47±0.26 vs. 0.78±0.15, DBP&STI = 0.62±0.25 vs. (0), and DBP&RFPI = (0) vs. (0). Although blood pressure and body composition phenotypes differ between the two studies, the heritabilities of the traits are similar in both. The genetic correlations between blood pressure and adiposity measures also are similar in the two studies, but there appear to be stronger genetic associations between blood pressure and fat patterning in the GHS families than in the FLS families. Supported by NIH grants HL19931 and HD12252.

Program Nr: 2253 from the 1999 ASHG Annual Meeting

Significant admixture linkage disequilibrium in African Americans across 30 cM around the *FY* locus. *M.W. Smith[1], J.A. Lautenberger[2], J.C. Stephens[3], S.J. O'Brien[2].* 1) IRSP, SAIC, Laboratory of Genomic Diversity, National Cancer Institute, Frederick, MD 21702-1201; 2) Laboratory of Genomic Diversity, National Cancer Institute, Frederick, MD 21702-1201; 3) Population Genomics Department, Genaissance Pharmaceuticals, Inc., New Haven CT 06511.

Understanding the importance of allelic polymorphisms on quantitative and environmentally interacting phenotypes is now turning to population association screens, especially in cases where pedigree analysis is difficult. Because association screens require linkage disequilibrium between markers and disease loci, maximizing the degree of linkage disequilibrium increases the chance of discovering functional gene-marker associations. One suggested approach, mapping by admixture linkage disequilibrium (MALD) using recently admixed African Americans, is evaluated here by measuring marker associations with 15 short tandem repeats (STRs) and an indel polymorphism of the AT3 locus in a 70 cM segment at 1q22-23 around the *FY* (Duffy) locus. The FY polymorphism (46T-C) disrupts the GATA promoter motif specifically blocking FY expression in erythroid cells and has a nearly fixed allele frequency difference between Caucasians and Africans which is likely a consequence of a selective advantage of *FY* -/- in malaria infections. The product of the *FY* gene is a chemokine receptor which functions as a scavenger of chemokines and no obvious deleterious phenotype of *FY* -/- individuals is currently known. Analysis of linkage disequilibrium around the FY gene revealed that four flanking loci (*D1S303, SPTA1, D1S2635* and *D1S484*) spanning 8 cM showed strong and consistent linkage disequilibrium with FY. Significant linkage disequilibrium signals were observed over a 30 cM region from 4.4 cM (*D1S2777*) to 16.3 cM (*D1S196*) for STRs and at *AT3* (25.3 cM), affirming the theory of MALD for genome scans and association analysis in population studies. This project has been funded in whole or in part with Federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. NOI-CO-56000.

Program Nr: 2254 from the 1999 ASHG Annual Meeting

In spite of being caused by a single point mutation, familial amyloid polyneuropathy (Val30Met) is not a simple Mendelian disorder. *A. Sousa[1,3,4], T. Coelho[2,3,4], L. Lobato[2,3,4], J. Sequeiros[1,4].* 1) Dept Population Studies, ICBAS; 2) HGSA; 3) CEP; 4) UnIGENe, IBMC, Porto, Portugal.

Although first described in Portugal by Andrade (1952), FAP is now known to be present in clusters in Japan, Northern Sweden, Majorca and Brazil, as well as in families of various origins. FAP is a motor, sensory and autonomic neuropathy, expressing in adulthood, and is inherited in an autosomal dominant mode. In all populations, a single point mutation (G for A) is responsible for the production of abnormal transthyretin, the precursor of amyloid.

Age-at-onset varies from an average of 33.5 yr. in Portugal to 56.7 yr. in Sweden (Majorcan patients show intermediate onset). However, within each of these clusters, some common patterns of variation can be observed: 1) late-onset and aged proven carriers cluster in some families, which seem to be more protected from the effects of the gene; 2) mean age-at-onset is higher in patients who had no affected parent than in patients with one affected parent; 3) anticipation of age-at-onset is significant. Although in the case of Swedish and Majorcan patients the sample size is small, in the case of Portuguese patients the anticipation study was performed in 770 parent-offspring pairs: it was significant (average 5.0 yrs) under different sampling schemes (exclusion of probands, exclusion of pairs with simultaneous onset, correction for cohort bias, all three together and a random sample).

We had previously postulated the possibility of a modifying gene of a dynamic-mutation type. So far, search for TNR were not conclusive. The number of studied twins (MZ vs. DZ) is not conclusive about the relative importance of genetic and environmental factors in age-at-onset. However, high correlations of age-at-onset in pairs of relatives (0.66 for sibs, 0.38 for parent-child in Portuguese population; 0.8 for sibs and parent-child in Majorcan population) point out to the importance of a genetic factor in regulating age-at-onset. Complex segregation analysis may yield some answers to the intriguing variability of an apparently simple genetic disorder.

Program Nr: 2255 from the 1999 ASHG Annual Meeting

Y-chromosome microsatellite haplotypes demonstrate presence of several components in male gene pool of Tuva population. *V.A. Stepanov*[1, 2], *V.P. Puzyrev*[1]. 1) Institute of Medical Genetics, Tomsk, Russia; 2) Tomsk State University, Tomsk, Russia.

Tuva is a native Siberian ethnic group living in Southern Siberia and speaking a language of Turkic branch of Altaic language family. Y-chromosome microsatellite haplotype analysis in three populations of Tuva reveals substantial intrapopulation variability in male Tuva population. In the sample of 111 chromosomes 49 different haplotypes were found, and frequency of only 4 of them was higher than 5%. The level of genetic diversity (H=0.935) demonstrate high discrimination power of Y-chromosome haplotypes. Analysis of molecular variance (AMOVA) and some other data testify for the absence of population subdivision in Tuva. Most of Tuva haplotypes fall in two lineages. Lineage A comprises ~64% of chromosomes and lineage B includes 24%. Presumable ancestral haplotype for lineage A is close to most common haplotype in modern Caucasians (Md=3), whereas ancestral haplotype for lineage B is distant from both "Caucasian" and "Mongolian" haplotypes. Age estimate for Y-chromsomes lineages in Tuva shows that male gene pool of modern Tuva population have its roots in upper Paleolithic or Neolithic periods. The computation of the age of Y chromosome lineages gives an estimate of 3500 years for lineage A (95%CI 1500 - 9180 years), and 5500 years for lineage B (95%CI 2360 - 14400 years). We suppose that lineage B corresponds to the ancient Indo-European contribution to the male gene pool of modern Tuva. More vast and less ancient lineage B may reflect the migration of ancient Turkic tribes to the territory of modern Tuva in Hun-Sarmatian period.

Program Nr: 2256 from the 1999 ASHG Annual Meeting

Vitamin D receptor polymorphisms and total hip bone mineral density in Baltimore women. *O.C. Stine, J.C. Scott, S. Zheng, D. Wiesch, K.M. Fox, E.R. Bleeker, M. Hochberg, D.A. Meyers.* Ctr Gen Asthma Complex Disease, Univ Maryland, Baltimore, MD.

Osteoporosis, a major cause of morbidity in the elderly, is a multifactoral disorder that has a substantial genetic component. In order to find genes associated with bone density, we have begun genetic studies of the women in the Baltimore cohort of the Study for Osteoporotic Fractures and their daughters. Total hip bone mineral density (BMD), a measure with significant heritability, was determined by DXA technology on 425 women (m = 75.3 years) and 55 of their daughters (m = 47.8 years). We obtained DNA samples and determined vitamin D receptor genotypes at the Fok I and Bsm I polymorphisms. Mothers and daughters were analyzed separately in order to avoid age as a confounding factor. When the average total hip BMD was compared between groups defined by their genotypes {Fok I, genotype = 1(uncut)1, n=135, $m_{BMD} = 0.75 \pm 0.13$; 12(cut), n = 158, $m_{BMD} = 0.75 \pm 0.13$; 22, n = 42, $m_{BMD} = 0.75 \pm 0.13$. Bsm I, genotype = 11, n = 97, $m_{BMD} = 0.74 \pm 0.13$; 12, n = 125, $m_{BMD} = 0.76 \pm 0.12$; 22, n = 120; $m_{BMD} = 0.74 \pm 0.13$}, no differences were found between the groups at either polymorphism. Of note, we observed, post hoc, that the Bsm I polymorphism was not in Hardy-Weinberg equilibrium in the mothers ($p < 0.0000005$). In contrast, the polymorphism is in Hardy-Weinberg equilibrium in the daughters. Inspection of the data for the mothers reveals that the cause of the disequilibrium is a deficit of heterozygotes. This may be because our sample is composed of several subpopulations, a Wahlund effect, or may be the result of selection against heterozygotes in older women. Additional information on the ethnicity of the mothers will be examined and a sample of elderly men will be genotyped to test these possible explanations. We conclude that there is no association between either VDR polymorphism and hip BMD in Caucasian women. We are continuing to study the relationship of other genetic polymorphisms to osteoporosis.

Program Nr: 2257 from the 1999 ASHG Annual Meeting

Estimation of admixture proportions in African-American and Hispanic populations of the continental US using highly polymorphic STR loci. *D.N. Stivers[1], R. Deka[2], B. Budowle[3], R. Chakraborty[1]*. 1) Human Gen Ctr, Sch Public Hlth, Univ Texas Health Sci Ctr, Houston, TX; 2) Dept. Environmental Health, Univ Cincinnati, Cincinnati, OH; 3) FBI Laboratory, FBI Academy, Quantico, VA.

It is well known that allele frequencies in admixed populations are weighted linear combinations of allele frequencies in the contributing parental populations, with the weights representing the admixture components from each parental population. Consequently, all allele frequency-based admixture proportions estimators are ratio-estimators whose depends upon the history of admixture and sampling uncertainties of the allele frequency estimates in the parental populations. A recently developed coalescence-based method of admixture estimation which exploits the natural of alleles at repeat loci by their repeat sizes apparently avoids such biases. The purpose of this research is to provide empirical support for this assertion.

We show that the logic of the coalescence-based method is mathematically equivalent to the least-square method based on decomposition of the variance of allele sizes in an admixed population. For illustration, we use allele size distributions in regional continental US populations of African-Americans and Hispanics in at nine short tandem repeat (STR) loci, for which allele frequency estimates are also available for Europeans, Africans and Native Americans. Estimates of admixture components (proportions of European, African and Native American genes) were obtained by least-squares, gene diversity and coalescence-based methods, along with the goodness-of-fit scores of the admixture models. The results support the earlier conclusions from protein polymorphism data suggesting bi-parental origin of African-Americans with regional differences in the proportion of Europen genes (increasing from South-East to Northwest). In contrast, the Hispanic populations of South-east and North-east appear to have a significant African contribution, which is not substantial in Western regions of the country.

(Research supported by US Public health Service research grants GM 41399, GM 53545 and GM 45861).

Program Nr: 2258 from the 1999 ASHG Annual Meeting

Polymorphisms in the IL-1 gene cluster and risk of osteoporotic fractures in elderly women. *K.L. Stone[1], S. van Dijk[2], H. Tabor[1], J. Cauley[1], K. Ensrud[1], E. Harris[1], J. Zmuda[1], M. Hochberg[1], J. Sorrell[3], G. Duff[3], S. Cummings[1].* 1) Study of Osteoporotic Fractures, San Francisco, Pittsburgh, Minneapolis, Portland, Baltimore; 2) Medical Science Systems, San Antonio; 3) Univ Sheffield, UK.

Polymorphisms in the interleukin-1 (IL-1) gene cluster have been associated with decreased bone mineral density in postmenopausal women. To test the hypothesis that these polymorphisms influence risk of osteoporotic fractures (FXs), we conducted a case-cohort study in a prospective study of 9,704 Caucasian women aged 65 yrs and older. FXs occurring since baseline over an average of 9.4 yrs were validated by X-ray. Incident spine FXs were determined by morphometry using X-rays collected at baseline and an average of 3.5 yrs later. Polymorphisms in the IL-1 gene cluster on chromosome 2 (IL-1A +4845, IL-1B +3954, IL-1B -511, IL-1RN +2018) were determined by PCR analysis of previously collected whole blood samples stored on filter paper. Genotypes were compared among women who suffered incident hip (n=216), spine (n=183) or wrist (n=216) FXs and controls drawn from a random cohort sample (n=626). Non-spine FX (n=185) risk was analyzed within the cohort sample. Results are reported as relative risks (RR). After excluding current or previous estrogen (ERT) users and adjusting for age, BMI, medical conditions, health status and other FX risk factors, the IL-1A 2,2 genotype was associated with an increased wrist FX risk (RR=1.8; 95% CI 1.0-3.5) relative to those with type 1,1, with similar patterns for non-spine (RR=1.6; 0.9-2.8) and spine (RR=1.4; 0.7-2.6) but not hip (RR=1.0; 0.5-2.2) FX. Non-spine FX risk was increased among those with IL-1B (+3954) genotype 2,2 (RR=2.0; 1.1-3.8), and decreased among those with IL-1RN genotype 2,2 (RR=0.4; 0.2-0.9). Different risk patterns were observed when ERT users were included, suggesting a possible gene-environment interaction. For example, in models including ERT users, women with IL-1RN genotype 2,2 experienced nearly double the risk of wrist fracture (RR=1.9; 1.1-3.3). Polymorphisms in the IL-1 gene cluster may influence risk of osteoporotic fracture in elderly women. Further study is needed to explain differences in patterns of association by fracture type.

Program Nr: 2259 from the 1999 ASHG Annual Meeting

Molecular Screening for Smith-Magenis Syndrome Among a Large Population of Patients with MR. *J.L. Struthers, S. Taylor, K. Harrison, M.M. Khalifa.* Department of Pathology and Pediatrics, Queen's University, Kingston, Ontario, Canada.

Smith-Magenis syndrome (SMS) is a MCA/MR syndrome caused by a microdeletion of chromosome 17p11.2. Clinical findings in SMS patients include facial anomalies, speech delay, behavioral problems and peripheral neuropathy. The incidence of SMS is estimated to be 1 in 25 000, however, it may be significantly underdiagnosed due its recent description and phenotypic variability.

In order to investigate the frequency of SMS among patients with undiagnosed MR, we have established a protocol to screen for this deletion in a large number of patients referred over a ten year period. DNA samples from these patients are digested with EcoRI and hybridized with the D17S258 probe--previously identified as the most commonly deleted region in SMS. This probe is non-polymorphic with EcoRI. Another EcoRI non-polymorphic probe on the X chromosome is included for a dosage control; males display a single dose with this probe, while females display a double dose. Detection of suspected SMS cases is by dosage comparison with the control probe using densitometry; individuals with the SMS deletion display only a single dose of the D17S258 band. Patients that are suspected as having the SMS deletion are selected and tested again. Samples that display a persistence of dosage difference are analyzed further. Highly polymorphic markers in the SMS region are then used to detect hemizygozity in these patients. Comparative Genomic Hybridization is also performed to identify the SMS deletion through comparisons with normal controls and known patients. Suspected patients are then requested to submit a new blood sample for conformation of the diagnosis by FISH.

To date, over 600 samples have been screened. Repeat testing has been performed in 14 cases; 9 exhibit persistence of the dosage difference. In 2 cases the dosage difference has been explained by a diagnosis of Klinfelter's syndrome. Conformation of SMS in the remaining cases is underway. This will be the largest study to identify SMS in patients with MR. The results will provide important insight into the phenotypic variability and frequency of this disease among individuals with undiagnosed MR.

Program Nr: 2260 from the 1999 ASHG Annual Meeting

The origin and genetic affinity of Sino-Tibetan speaking populations. *B. Su[1], R. Deka[2], C. Xiao[1,3], J. Xiao[4], P. Underhill[5], L. Cavalli-Sforza[5], R. Chakraborty[1], L. Jin[1,4].* 1) Human Genetics Center, Univ Texas Sch Pub Hlth, Houston, TX; 2) Department of Environmental Health, University of Cincinnati, Cincinnati, OH; 3) Department of Biology, Yunnan University, Kunming, China; 4) Institute of Genetics, Fudan University, Shanghai, China; 5) Department of Genetics, Stanford University, Palo Alto, CA.

Among over 6,000 languages currently spoken in the world. The Sino-Tibetan language family is the largest of all in view of population size (more than 1 billion speakers), including 360 languages spoken in nine Asian countries, China, India, Bhutan, Myanmar, Bangladesh, Laos, Thailand and Vietnam. By using 19 Y chromosome biallelic markers and 3 Y chromosome microsatellite markers, we analyzed the genetic structures of 20 indigenous Sino-Tibetan speaking populations from East, Southeast, and South Asia. Our results showed that H6 (defined by a T to C mutation at M122 locus) and H8 (defined by a G deletion at M134 locus) are the predominant haplotypes in almost all Sino-Tibetan populations, implying the genetic affinity of populations in the same language family. Furthermore, the clinal distribution of H8 suggest that an ancient westward and then southward migration that resulted in the peopling of the Himalayan areas, including Tibet, Northeast India and North Myanmar. We therefore postulate that the ancient Qiang people living in Upper Yellow River basin in 7000 years ago, who developed the earliest Sino-Tibetan civilization, might be the ancestor of modern Sino-Tibetan populations.

Program Nr: 2261 from the 1999 ASHG Annual Meeting

Population genetics of the SCA6 locus in British families with ADCA. *M.G. Sweeney, P. Giunti, V.J. Stinton, P.F. Worth, M.G. Hanna, N.W. Wood, M.B. Davis.* Clinical Neurology, Institute of Neurology, London, UK.

An expanded CAG repeat in the voltage-gated calcium channel subunit (CACNA1A) has been shown to cause an autosomal dominant, predominantly late onset, 'pure' cerebellar ataxia (SCA6). We have studied this mutation in 70 families with autosomal dominant cerebellar ataxia (negative for SCAs 1, 2, 3 and 7), of these 35 have been shown to be have an expansion at this locus. All affected individuals in this population had 22 or 23 repeats. Other reports have found expansions ranging from 21 to 30 repeat units. In 9 phase known families (of which 6 had 22 repeats) haplotype analysis using markers which closely flank the repeat unit and an intragenic marker (D19S1150) were studied. All individuals with 22 repeats shared a common allele for the intragenic marker and one or more flanking markers. One individual with 23 repeats also shared this common haplotype. These data suggest that all chromosomes with the repeat expansion appear to have derived from a small number of ancestral chromosomes and provide evidence of a founder effect. Of the 3 remaining families with 23 repeats, 2 shared a haplotype that differed from those with 22 repeats at all markers studied so far. The remaining family showed clear evidence for a new mutation at the SCA6 locus, in which the expansion occurred on a maternal chromosome with 13 repeats.

Program Nr: 2262 from the 1999 ASHG Annual Meeting

Epidemiological, genetic and etiologic study of the congenital and infantile cataract in Colombia. *M.L. Tamayo[1], A. Ordoñez[1], C.M. Leyva[1], J.C. Prieto[3], M. Lozano[2], J.C. Serrano[4], C. Peñaranda[4], P. Garavito[4], S. Scaff[4], F. Escallon[4], F. Vejarano[4], A. Cortez[4], F. Betancourt[4], P. Echeverry[4], O. Alvis[4], E. Gutierrez[4], A.M. Alvarez[4].* 1) Unidad de Genetica Clinica, Univ Javeriana, Instituto de Genetica Humana. Bogota, Colombia; 2) Fundacion Oftalmologica Nacional. Bogota, Colombia; 3) Laboratorio de Genetica, Hospital La Victoria. Bogota, Colombia; 4) Several eye institutions in Colombia.

Cataracts are one of the most common causes of subnormal vision and blindness. We evaluated a series of individuals with congenital and infantile cataract from 9 Colombian cities in order to determine its etiology (genetic or acquired) and to analyze the diverse epidemiological factors in our country. We selected 264 individuals with confirmed diagnosis of congenital and infantile cataract. A complete physical, ophthalmologic and genetic evaluation was performed to each individual and available family members. The clinical history included a detailed analysis of family and personal background and data regarding important epidemiological aspects. Samples were taken to perform the following laboratory examinations: general metabolic - biochemical studies in blood and urine (qualitative tests in biochemical genetics, aminoacid and carbohydrate chromatography), galactose-1-Uridyl transferase enzyme activity, antibodies against toxoplasm and rubella, and karyotype. The karyotype analysis showed a cytogenetic abnormality in 8.46% of the individuals. A high frequency of microphthalmus and other ocular associations with cataract was observed. The genetic cases correspond to the highest frequency (58%), followed by not defined (30%) and acquired (12%). It was established that the autosomal dominant congenital cataract was the most frequently encountered and in those families an inter and intrafamilial clinical variability was observed.

Program Nr: 2263 from the 1999 ASHG Annual Meeting

Extent of confounding odds ratio in case control association studies due to population stratification. *M.D. Teare[1], A.M. Dunning[2], C.S. Healey[2], F. Durocher[1], D.F. Easton[1], B.A.J. Ponder[2], N.E. Day[3].* 1) CRC Genetic Epidemiology Unit, University of Cambridge, Cambridge, England, United Kingdom; 2) CRC Department of Oncology, University of Cambridge, UK; 3) Department of Community Medicine, University of Cambridge, UK.

Population stratification is often cited as a problem when conducting case-control genetic association studies. If stratification exists, then cases may be drawn from different sub-populations to the controls. If these populations experience different rates of disease, marker genotypes may appear associated whenever sub-population marker allele frequencies are sufficiently different. This could result in confounding odds ratios which are greater than 1.

We have attempted to evaluate the extent of such confounding association in a series of 700 control from East Anglia, UK. In a pair-wise association analysis of six unlinked loci, only one pair (out of 15 possible) showed significant association. At this stage, therefore, we have no evidence of stratification in this population. We have formulated a random effects model that provides a powerful framework within which to test for stratification utilising an unlimited number of unlinked marker loci. Using this approach, for any association detected between marker and disease it is possible to estimate what fraction of this could be due to stratification alone. We have also investigated under what circumstances such stratification can be detected. For example, a sample size of 1000 should be sufficient to detect any stratification causing a confounding odds ratio of 1.3 or more, assuming that the disease is no more than twice as common in one stratum than the other. Failure to detect associations between unlinked markers should therefore protect against spurious associations unless there is extremely strong heterogeneity of risk.

Program Nr: 2264 from the 1999 ASHG Annual Meeting

Evaluation of DNA isolated from mouthwash for genetic studies. *L.H. Toji, E.A. Morgan, P.K. Bender, J.C. Beck.*
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In order to evaluate the suitability of the simple mouthwash procedure described by Lum and LeMarchand [*Cancer Epidemiol. Biomarkers Prev.* **7:** 719-724 (1998)] for collection of DNA for genetic/epidemiological studies, samples were collected with informed consent from dental patients at the time of dental examination and from several inhouse laboratory staff.

The DNA was isolated with the Qiagen Blood Kit, quantitated by SYBR green fluorescence and characterized by routine and pulsed-field agarose gel electrophoresis. Yields of DNA ranged from 0.6 to 8 micrograms and the size varied from 20 to 40 kb. The DNA was suitable for PCR-based assays such as: (a) the allelic discrimination assay for gender using the amelogenin locus, (b) genotyping with a set of six microsatellite markers and with the chromosome 3 and 22 portions of the ABI mapping panel II recommended for genome scanning, (c) the detection of trinucleotide repeats in the normal range of the Huntington disease locus and (d) long PCR (Calm-1, 5.6 kb).

Because the yields of DNA are very variable and not always sufficient for detailed analyses, three samples, ranging from the lowest to highest yields, were also examined as template for whole genome amplification (WGA) [Zhang *et al., Proc. Natl. Acad. Sci., USA*, **89:** 5847-5851 (1992)] to determine if the available quantities of these samples could be extended. The whole genome amplified product exhibited the same microsatellite profile with the six tetranucleotide repeat microsatellite markers and the panel of dinucleotide repeat markers as the DNA before WGA while these microsatellite markers were not detectable in comparable dilutions of the mouthwash DNA without WGA. These results attest to the utility of DNA isolated from mouthwash samples for genetic studies.

Program Nr: 2265 from the 1999 ASHG Annual Meeting

Cleft lip and palate anomalies in South America. A case-control study of gene-environment interactions targeted on primary prevention. *M. Tolarova[1], L. Pastor[2], S. Tolarova[3], A. Goldberg[4], M. Guinazu[2], T. Mosby[1], M. Pastor[2], J. Mir[1], A. Capozzi[5]*. 1) Prg for Cleft Prevention, Univ California, San Francisco, CA; 2) University of Cuyo,Mendoza, Argentina; 3) Veteran's Hospital, University of Minneapolis, MN; 4) Marin General Hospital, San Rafael, CA; 5) Shreiners Hospital, Sacramento, CA.

Cleft lip and palate anomalies represent a major problem in oral health worldwide.Every two minutes in this world one child with cleft is born-660 every day and 235,000 each year.With a projected population growth of 1.8 million/year, an additional 3,200 cleft children will be born every year in the future unless we invest our scientific knowledge and finances into prevention. At present, 7,500 children with cleft are born every year in the USA and at least 13,000 in South America (SA).In SA, 25-30% of individuals with cleft will never have surgery. With the projected population growth, the numbers of affected individuals will increase dramatically in future years.Our case-control study is focused on identifying genetic and environmental factors that contribute to the high prevalence of clefting in 5 locations in SA.Altogether, data from interviews of mothers of 1334 cases and 981 controls have been collected and blood specimens for genotyping and for blood levels of folates and other vitamins were obtained from chilld-mother pairs.Preliminary results from one location showed a high prevalence of 677C®T mutation of MTHFR gene in cases (18.3%)compared to controls(5.7%),but low plasma and red blood cell levels of folate and a dietary pattern that is lacking sufficient sources of folate in both cases as well as in controls. Other locations, however, showed significant differences between the nutritional pattern of cases and control mothers.Based on these preliminary results we extrapolate that among environmental factors,nutrition will always play an important role.It will be necessary to develop a different preventive aproach based on local data.

This study was initiated by the Program for Prevention of Cleft Lip and Palate/Craniofacial Anomalies, UCSF and supported by Rotaplast Intl., Inc.

Program Nr: 2266 from the 1999 ASHG Annual Meeting

A population-based survey reveals an extremely high FMF carrier frequency in Armenia, suggesting heterozygote advantage. *Y. Torosyan[1], I. Aksentijevich[1], T. Sarkisian[2], V. Astvatsatryan[3], A. Ayvazyan[4], M. Centola[1], J.J. Chae[1], D.L. Kastner[1]*. 1) Dept Arthritis & Rheumatism, NIAMS/NIH, Bethesda, MD; 2) Department of Genetics, Yerevan State University; 3) Department of pediatrics, Yerevan Medical University; 4) Erebuni Medical Center, Yerevan, Armenia.

Familial Mediterranean fever (FMF) is a hereditary inflammatory disease characterized by self-limited recurrent episodes of fever and aseptic serosal and synovial inflammation, mostly occurring in Armenians, Jews, Turks, and Arabs. Some patients also develop systemic AA amyloidosis. Among 201 independent carrier chromosomes from a cohort of 111 affected individuals, 91% were positive for a panel of 13 mutations, and 80.6% carried 1 of 3 common mutations. In a single family a novel R42W mutation was identified. M694V was the most frequent mutation, but M680I (20.4%) and V726A (23.4%) demonstrated comparable levels. Comparisons between the frequencies of these 3 mutations among affected individuals and in the general population suggest that M680I has the highest penetrance. Among the mutations outside of exon 10, where a majority (90.8%) of the identified mutations clustered, F479L (exon5) appears to have the highest penetrance. Analysis of 10 FMF patients with amyloidosis revealed a spectrum of mutations, with M694V most common (55%) but with substantial contributions from other mutations (M680I 30%, V726A 10%, R761H 5%). Unlike all other mutations, P369S (exon 3) was more frequent in controls (9.95% in 392 chromosomes) than among affected individuals (2.0% of 201 alleles), suggesting a possible protective effect. The total carrier frequency (2pq) in Armenia, derived by screening for 9 common MEFV mutations, is about 1:2. Based on the frequencies the 3 high-penetrance exon 10 mutations (M680I, M694V, V726A), the disease frequency may be as high as 1:25. Comparison with a 1993 population-based estimate of 1.13% suggests incomplete penetrance even for these mutations. Nevertheless, these data indicate the highest frequency of MEFV mutations measured to date, and underscore the possibility that some of these mutations may confer an as yet unidentified heterozygote advantage.

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Program Nr: 2267 from the 1999 ASHG Annual Meeting

Genetic regulation of skeletal maturation from 3 to 15 years. *B. Towne[1], R.M. Siervogel[1], J.S. Parks[2], M.R. Brown[2], A.F. Roche[1], J. Blangero[3].* 1) Wright State University School of Medicine, Dayton, OH; 2) Emory University School of Medicine, Atlanta, GA; 3) Southwest Foundation for Biomedical Research, San Antonio, TX.

We used a multivariate variance components method incorporating parametric correlation functions to model the heritability of skeletal maturity in healthy children aged 3 to 15 years, and the genetic and environmental correlations between skeletal maturity assessed across this age range. A total of 6,893 annual skeletal age (SA) assessments from hand-wrist radiographs, collected since 1932 as part of the Fels Longitudinal Study, from 807 children in 192 kindreds, were simultaneously analyzed. SA was assessed using the FELS method, a maximum likelihood procedure that estimates a child's SA at their current chronological age. Models positing a stable heritability of SA from chronological ages 3 to 15 years, and models positing constant genetic and/or environmental correlations between SAs across this age range, were rejected. The best fitting model had 65 parameters and allowed for an exponential decay in genetic and environmental correlations as a function of chronological age differences. The heritabilities of SA at each chronological age were: 3=0.71, 4=0.73, 5=0.77, 6=0.93, 7=0.78, 8=0.77, 9=0.73, 10=0.63, 11=0.45, 12=0.39, 13=0.34, 14=0.23, and 15=0.11. The genetic correlation matrix showed a pattern of decreasing correlations between SA at different chronological ages as age differences increased (e.g., the correlation between SA at age 3 and SA at age 4 was 0.96, but was 0.56 between SA at age 3 and SA at age 15). The environmental correlation matrix showed a more pronounced pattern of decreasing correlations between SA at different chronological ages as age differences increased (e.g., the correlation between skeletal age at age 3 and skeletal age at age 4 was 0.77, but was 0.12 between skeletal age at age 3 and skeletal age at age 15). These results show a significant heritability of skeletal age through puberty, and suggest that there are developmental stage-specific genetic and environmental influences on skeletal maturation. Supported by NIH grants HD12252, HD31621, MH59490, and HD36342.

Program Nr: 2268 from the 1999 ASHG Annual Meeting

Apolipoprotein E and morbidity in an elderly population. *C.M. Van Duijn[1], A.J.C. Slooter[1], M. Cruts[2], M.M.B. Breteler[1], P.V.T.M. De Jong[1], H.A.P. Pols[1], J.C.M. Witteman[1], C. Van Broeckhoven[2], A. Hofman[1]*. 1) Epidemiology & Biostatistics, Erasmus Univ Medical Sch, Rotterdam DR, Netherlands; 2) Neurogenetics Laboratory, Flanders Interuniversity Institute for Biotechnology, Born-Bunge Foundation, University of Antwerp (UIA), Belgium.

The apolipoprotein E gene (APOE) plays an important role in lipid metabolism. APOE also is a genetic determinant for Alzheimer's disease (AD), an important cause of mortality in the elderly. APOE has been reported to be a gene determining longevity. We studied the impact of the APOE gene on morbidity and mortality in a population-based study of 5290 subjects aged 55 years or older (the Rotterdam Study). Subjects were followed 4.0 years. Up to age 65 years, subjects of the APOE44 genotype had a significantly higher risk of myocardial infarction than carriers of the APOE33 genotype. No effect of APOE was found on the risk of myocardial infarction at old age. The risk of stroke was not significantly related to APOE. Although carriers of the APOE*4 allele were at increased risk of AD, the contribution of APOE*4 to the development of AD at the population level was modest. Each year, an estimated 185 per 100000 individuals aged 55 years or over were estimated to become demented due to carrying the APOE*4 allele. As an association of APOE to longevity would predict a change in allele frequency, we assessed APOE allele frequencies by age. The APOE*4 allele frequency decreased from 0.17 in 55-years old to 0.12 in subjects 90 years or over. The decrease in APOE*4 was to the benefit of APOE*3 because also the frequency of the APOE*2 allele slightly decreased with age. Our findings suggest that the APOE genotype does not play a major role in mortality in the elderly.

Program Nr: 2269 from the 1999 ASHG Annual Meeting

Analysis of the C677T and A1298C mutations of the MTHFR gene as risk factors for spina bifida. *K.A. Volcik[1], S.H. Blanton[2], G.H. Tyerman[3], S.T. Jong[3,4], E.J. Rott[4], R.J. Mier[3], N.K. Romaine[1], H. Northrup[1]*. 1) Div Medical Genetics, Univ Texas Houston Med School, Houston, TX; 2) Dept of Ped, Univ Virginia, Charlottesville, VA; 3) Shriners Hospitals for Children- Houston, Los Angeles and Lexington; 4) Dept of Ped, Baylor College of Medicine, Houston, TX.

The C677T and A1298C mutations in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene are each associated with reduced MTHFR activity. The C677T mutation in the heterozygous and homozygous state correlates with increased enzyme thermolability, with homozygous mutant genotypes showing significantly elevated plasma homocysteine levels and decreased plasma folate levels. Changes in neither homocysteine nor folate levels are associated with heterozygous or homozygous A1298C variant genotypes. However, there is an interaction between these two variants such that combined heterozygosity for the C677T and A1298C variants results in even lower MTHFR activity than heterozygosity for either of the MTHFR mutations separately, thus resulting in significantly elevated homocysteine and decreased plasma folate levels. Our study determined the frequencies of the C677T and A1298C MTHFR mutations for spina bifida (SB) cases, their parents and controls in two ethnic populations: Hispanics of Mexican-American descent and North American non-Hispanic whites. Our populations were further categorized as to whether the spina bifida lesion occurred as an upper or lower level defect, according to the Van Allen multi-site closure model. Hispanic SB cases with upper level defects and their mothers were homozygous for the C677T variant allele at a significantly higher rate than the Hispanic control population [OR=2.05,p=0.03; OR=2.62,p=0.01, respectively]. Non-Hispanic white SB cases with lower level defects have a greater frequency of the compound heterozygous C677T/A1298C genotype [OR=2.78,p=0.01]. Due to our current findings, we postulate that the C677T MTHFR homozygous mutant genotype is a risk factor for upper level spina bifida defects in Hispanics, while the combined C677T/A1298C heterozygous genotype is a risk factor for lower level spina bifida defects in non-Hispanic whites.

Program Nr: 2270 from the 1999 ASHG Annual Meeting

Familial aggregation of carotid atherosclerosis in sibling pairs with type 2 diabetes. *L.E. Wagenknecht, B.I. Freedman, W. Riley, D.W. Bowden.* Wake Forest Univ Sch of Med, Winston-Salem, NC.

Type 2 diabetes is a major risk factor for atherosclerotic cardiovascular disease. A subclinical measure of atherosclerosis is the intimal medial thickness (IMT) of the common carotid artery (CCA). The role of genetic factors that contribute to CCA IMT is controversial, with heritability of CCA IMT ranging from 10% - 80%. We hypothesize that type 2 diabetes, commonly characterized by obesity and dyslipidemia, accelerates atherosclerosis at a rate dependent, in part, upon genetic factors.

A total of 53 diabetic relatives in 24 families were studied for extent of atherosclerosis (CCA IMT measured by B-mode ultrasound), as well as lipids, blood pressure and other risk factors using a standardized questionnaire. These data were analyzed by univariate and birariate variance components using the SOLAR (Almasy & Blangero) software package. Two strong risk factors (phenotypes) were chosen for the focus of this analysis -- CCA IMT and HDL. In this group of sibships, the mean ($\pm$ se) CCA IMT was 0.663 $\pm$ 0.015 mm and HDL level was 37.5 $\pm$ 1.8; mean age was 59.6 $\pm$ 1.2 yrs, duration of diabetes 9.8 $\pm$ 1.1 yrs, and BMI 32.5 $\pm$ 1.9.

Univariate analyses suggested that CCA IMT was strongly heritable (0.84 $\pm$ 0.36, $p < 0.008$), as was HDL (0.55 $\pm$ 0.39, $p < 0.07$). Using a bivariate analysis of CCA IMT and HDL, the estimated heritability of CCA IMT was 0.71 ($p < 0.001$), of HDL was 0.35 ($p < 0.05$). The genetic correlation between CCA IMT and HDL was 0.15, and the environmental correlation between CCA IMT and HDL was -0.21. These results suggest that strong (but independent) genetic factors contribute to the variance of CCA IMT and HDL, with relatively small contributions to the variance due to measured covariates. In these data, both CCA IMT and HDL are highly heritable in sibling pairs concordant for type 2 diabetes, and may serve as an important feature in designing studies to map genes contributing to atherosclerosis and diabetic macrovascular disease.

Program Nr: 2271 from the 1999 ASHG Annual Meeting

Cloning of primate P450c17 cDNAs from genomic DNA: the molecular evolution of adrenarche. *J.T. Wang, W. Arlt, J.W.M. Martens, W.L. Miller.* Pediatrics, UC San Francisco, San Francisco, CA.

The CYP17 gene encodes a single form of cytochrome P450c17 that catalyzes two distinct, independently post-translationally regulated reactions: 17a-hydroxylation of pregnenolone (preg) or progesterone (prog) to 17OH-preg or 17OH-prog, and 17,20 lyase activity converting 17OH-preg to dehydroepiandrosterone (DHEA). Children produce very little DHEA, but at adrenarche at age 7-10 years DHEA production increases reaching maximum levels at 25-35 years which then wane slowly to childhood levels at > 70 years. The chimpanzee and gorilla have similar developmental DHEA patterns, but other primates including the rhesus monkey and baboon show a continuous high secretion of DHEA throughout life. To identify sequences important for post-translational regulation of human 17,20-lyase activity, we cloned and sequenced the P450c17 genes and cDNAs from rhesus monkey, baboon, and chimpanzee and compared them to our previously cloned human sequences. The rhesus cDNA was cloned by RT-PCR of adrenal RNA, but baboon and chimp adrenals were not available. Therefore, we used human oligos to amplify the full-length 6.5 kb chimp and baboon genes from leukocyte DNA and subcloned these into CMV-based pcDNA3 vector, and expressed these in COS-1 cells. The chimp and baboon P450c17 cDNAs were then PCR-amplified from the transfected cells; their sequences were correctly spliced and were identical to the sequences of each exon determined by PCR from the original genomic DNA. The human sequence was 99.2% identical to chimp, 96.5% identical to baboon and 96.1% to rhesus. Of 29 non-identical amino acids across the four species, 19 were differences between the higher (human, chimp) and the lower (baboon, rhesus) primates. These 19 amino acids included 3 Thr and 3 Ser residues in the higher primates, but only 2 Thr and one Ser in the lower primates. This may influence the Ser/Thr phosphorylation of P450c17 that is required for human 17,20-lyase activity (PNAS 92: 10619). Thus the sequence identities among these four species of P450c17 correlate well with the presence of adrenarche.

Program Nr: 2272 from the 1999 ASHG Annual Meeting

Lack of association between the a**2-macroglobulin polymorphism and Alzheimer's disease.** *X. Wang*[1], *S.T. DeKosky*[1,2], *M.I. Kamboh*[1,2]. 1) Department of Human Genetics, Univ Pittsburgh, Pittsburgh, PA; 2) Deparment of Psychiatry, Univ Pittsburgh, Pittsburgh, PA.

Alzheimer's disease (AD) is a complex and multifactorial disease involving many genetic and environmental factors. Among the genetic factors, the apolipoprotein E (APOE) gene has been identified as a susceptibility gene for AD. In addition, a2-macroglobulin (A2M) has been implicated in pathogenesis of AD. A2M is upregulated in brain during injury. In AD patients, A2M has been localized immunochemically to senile plaques. *In vitro*, A2M binds to and attenuates the propensity of Ab peptide to form neurotoxic fibrils. Recent association studies indicate the potential involvement of A2M in affecting the risk of AD. However, unlike APOE, this association has not been well established. This study was undertaken to investigate the role of the A2M intronic 5-bp deletion polymorphism in the development of AD and to define the interaction between APOE and A2M. The A2M polymorphism was screened using a PCR based assay in 567 white cases of late-onset AD cases and 319 controls. The distributions of A2M genotype (p=0.35) and allele (p=0.68) frequencies were comparable between cases and controls. Although the frequency of the A2M/DD genotype was higher in cases than controls, the difference was not statistically significant (3.6% vs. 1.9%; p=0.16). When we divided our data by *APOE*4* status, no significant difference was found between cases and controls either. Logistic regression analysis did not reveal any significant interaction between the APOE and A2M genes in affecting the risk of AD (p=0.19). In conclusion, our study indicates that there is no association between the intronic polymorphism of A2M and AD.

Program Nr: 2273 from the 1999 ASHG Annual Meeting

Increased prevalence of chronic rhinosinusitis in CF mutation carriers. *X.J. Wang, G.R. Cutting.* Institute of Genetic Medicine, Johns Hopkins Univ Sch Med, Baltimore, MD.

Chronic rhinosinusitis is one of the most prevalent of the chronic diseases, affecting about 15% of the U.S. population. It is an almost invariable feature of Cystic Fibrosis (CF). To evaluate whether CFTR plays a role in isolated rhinosinusitis in the general population, we initiated a CFTR gene mutation analysis in the patients with chronic rhinosinusitis. Screening of 147 Caucasian patients identified 11 patients with a CF mutation. Nine had the common mutation, DF508, one had G542X, and one had N1303K. Two DF508 carriers were identified in the 123 Caucasian controls. DGGE and sequencing of each CFTR coding region revealed that one of the eleven patients carried a second CF mutation (2789+5G®A). Sweat chloride testing and nasal potential difference (NPD) measurement indicated that only the patient with 2 CF mutations had CF. Ten CF mutation carriers among 146 patients (7%) is significantly higher than the rate in the control group (P=0.03 fisher exact). If 7% of chronic rhinosinusitis patients are carriers of a CF mutation, we estimate that about one third of CF carriers in Caucasians should have chronic rhinosinusitis. To evaluate this hypothesis, we surveyed the parents of CF patients who are obligate CF mutation carriers in 2 stages. In the first step, we asked participants whether they ever had rhinosinusitis, and, if so, the frequency and duration. Among 190 responses, 60% reported positively and 19% of the responses met established criteria for the chronic rhinosinusitis. As a control, we asked participants whether they ever had back pain. Chronic back pain has a prevalence of 18% in U.S. adults. Among 190 responses, 69% reported positively but only 13% met criteria for chronic back pain. In the second phase, a detailed questionnaire for historical evidence of chronic rhinosinusitis was sent to every participant. In 122 returns, 46 (38%) met the diagnostic criteria for chronic rhinosinusitis. The 38% chronic rhinosinusitis rate in CF carriers was significantly higher than the 15% prevalence in the U.S. population ($P=1 \times 10^{-4}$, c^2 test). Carrying a single CF mutation is a risk factor for the development of chronic rhinosinusitis.

Program Nr: 2274 from the 1999 ASHG Annual Meeting

Frequency analysis of autosomal dominant cerebellar ataxias at Ibaraki prefecture in Japan. *M. Watanabe[1], M. Kanemoto[4], N. Ohkoshi[1], A. Tamaoka[1], A. Hayashi[1], T. Yoshizawa[1], A. Mochizuki[1], H. Yoshida[1], S. Matsuno[1], K. Ishikawa[2], H. Miura[3], S. Shoji[1]*. 1) Neurology/Inst Clinical Sci, Tsukuba Univ, Tsukuba, Japan; 2) Neurology/Tokyo Medical and Dental Univ, Tokyo, Japan; 3) 5th department of Internal Medicine/Tokyo Medical Univ, Ibaraki, Japan; 4) School of Medicine/Tsukuba Univ, Tsukuba, Japan.

Purpose : To obtain a estimate of the relative frequency of spinocerebellar ataxia (SCA) type 1, 2, 3, 6, 7, 8, and DRPLA among ataxia families at Ibaraki prefecture in Japan. Materials and methods : Thirty two unrelated kindreds with ADCA were available for frequency analysis of ADCAs. Genomic DNA was extracted from peripheral blood samples of these ADCA patients with informed consent. Polymerase chain reaction amplification of the CAG repeat was performed using FITC labelled gene specific primer sets. PCR products were separated by electrophoresis with an autoread sequencer (ALFII, Pharmacia), and their sizes were determined using the associated software (Fragment Manager, Pharmacia). In the case of ADCA type III, ADCA type I, and progressive myoclonus epilepsy, we checked SCA6, SCA3, and DRPLA mutation for the first place respectively. Then we screened sequentially for the SCA1, SCA2, SCA3, SCA6, SCA7, SCA8, and DRPLA CAG (CTG for SCA8) expansion until an expansion at one of these ataxia loci was identified in the proband. Results : Among the 32 dominant kindreds, we found SCA6 expansion at a frequency of 45%, SCA3(MJD) expansion at a frequency of 34%, SCA2 at a frequency of 9%, and DRPLA at a frequency of 6%. No patients in our population tested were positive for SCA1, SCA7, and SCA8. SCA6 presented with predominantly cerebellar syndrome and patients often had onset after 40 years of age. SCA3(MJD) patients frequently developed ophthalmoplegia, spasticity, dystonia, and muscular atrophy, apart from ataxia. In SCA6 and SCA3(MJD), the CAG repeat lengths correlated inversely with age at onset. Conclusion : SCA6 is most prevalent in our population, while SCA3(MJD) is most prevalent in the other area of Japan.

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Program Nr: 2275 from the 1999 ASHG Annual Meeting

Sequence polymorphism and divergence in the human b-**globin gene cluster.** *M.T. Webster, R.M. Harding, J.B. Clegg.* MRC Molecular Haematology Unit, Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford, OX3 9DS, UK.

In an extension of our analyses of variability in the b-globin gene, we have examined levels of DNA sequence diversity and human/chimp divergence in 3 further loci within the human b-globin gene cluster, using samples from a Kenyan population. These loci lie within a 12.3 kb region 5' to the b-globin gene, separated from it by a hotspot of recombination. In order of increasing distance from the hotspot (3' to 5') they are the d-globin gene, a 3 kb intergenic region (known as the R/T) and the b-globin pseudogene. The d-globin gene is a functional adult globin gene, expressed at relatively low levels, the R/T region has no known function and the pseudogene is thought to have been inactivated early in primate evolution. These 3 loci each show the same levels of divergence between human and chimp, consistent with the b-globin gene, situated on the other side of a recombination hotspot, and also with average divergence for autosomes. Nucleotide polymorphism, measured as the number of segregating sites in our sample of 46 chromosomes, is also evenly apportioned among loci. Contrary to neutral expectations, neither levels of divergence nor diversity are greater in the b-globin pseudogene compared with the b-globin gene. One striking anomaly becomes apparent when we compare the 3 loci for nucleotide diversity measured as heterozygosity (i.e. from average pairwise sequence difference). Compared with the b-globin gene and pseudogene, nucleotide heterozygosity is reduced in d-globin and substantially higher in the R/T region. These patterns in the heterozygosity indicate differences in haplotype structure among loci and are consistent with the presence of two highly divergent alleles at the R/T locus in human populations (observed by Maeda *et al.* in 1983). By comparison, the b-globin pseudogene has several related haplotypes and the d-globin locus has a single major allele. These data imply that linkage disequilibrium varies greatly within the b-globin gene complex as a consequence of the evolutionary interplay of recombination, mutation and genetic drift.

Program Nr: 2276 from the 1999 ASHG Annual Meeting

Haplotype based diagnosis of 3-Methylglutaconic aciduria type 3 (Costeff syndrome). *Y. Weigl[1], E. Gak[1], D. Lev[2,3], L. Peleg[1,3], B. Goldman[1,3], M. Frydman[1,3].* 1) Medical Genetics, Danek Gertner Institute, Tel Hashomer, Israel; 2) Medical Genetics, Wolfsaon Hospital Holon; 3) Sackler School of Medicine, Tel Aviv University, Israel.

Costeff syndrome is an autosomal recessive condition characterized by infantile optic atrophy, an early onset, spastic paraplegia, mild ataxia, mild cognitive deficiency, dysarthria and 3-methylglutaconic aciduria. Recently linkage disequilibrium with the myotonic dystrophy protein kinase gene on 19q was reported in Iraqi Jews (Nystuen et al, Hum Mol Genet 1997 6:563-9). Microsatellites ERRC2CA6 and D15S908 are tightly linked to the disease locus, and allele # 1 of both microsatellites is in full linkage disequilibrium with the mutation. Using these microsatellites we studied 6 families at risk and 37 unselected and unrelated individuals of Iraqi Jewish origin. None of the 74 chromosomes of unrelated individuals carried allele 1 of markers ERRC2CA6 and D15S908. In addition 10 normal chromosomes of obligatory carries and 4 chromosomes of distant relatives were studied and were not carrying allele # 1 in either locus. In one mating of a disease haplotype carrier and his unrelated spouse, a healthy offspring, homozygous to allele # 1 at both loci was found. This individual either carries the background haplotype or an historic recombinant haplotype. In the 6 families reported by Nystuen et al, 13 normal chromosomes were found, neither carrying a disease associated allele at the two loci we studied. The prevalence of the disease haplotype in the Iraqi Jewish population is < 1/74. The prevalence of the background haplotype is 1/ 113. With a prevalence of < 0.01 for either allele, the prevalence of the haplotype should be < 0.0001. Thus, the detection of this haplotype in an individual of Iraqi origin is almost certainly an indication that this individual carries a 3MGA mutation. In the 6 families at risk, all the affected individuals were homozygotes to the 1-1 haplotype. Two singelton and one twin pregnancies were monitored. 3 fetuse were heterozygote carriers and one a homozygote normal.

Program Nr: 2277 from the 1999 ASHG Annual Meeting

Association of the interleukin-1 receptor antagonist IL1RN*1 allele with systemic lupus erythematosus (SLE) clinical phenotypes. *A. Weinreb[1,2], J.M. Grossman[2], R.M. Cantor[3], N. Theophilov[2], D.J. Wallace[4], B.H. Hahn[2], B.P. Tsao[2]*. 1) Division of Rheumatology, VA GLAHS, Los Angeles, CA; 2) Division of Rheumatology,UCLA, Los Angeles, CA; 3) Division of Human Genetics, UCLA, Los Angeles, CA; 4) Cedars-Sinai Medical Center, Los Angeles, CA.

SLE is a complex autoimmune inflammatory disease characterized by autoantibody production in which multiple genetic and environmental factors contribute to its pathogenesis. Interleukin-1 receptor antagonist (IL1RN), a competitive inhibitor of interleukin-1 (IL-1) that downregulates inflammation, may contribute both to SLE susceptibility and severity. The IL1RN*1 allele has previously been associated with SLE susceptibility and severity in both English and Swedish cohorts. We tested SLE clinical phenotypes in a North-American Caucasian cohort of 72 unrelated individuals for associations with IL1RN alleles. The SLE clinical phenotypes tested included malar rash, discoid rash, serositis, nephritis, hematologic abnormalities (hemolytic anemia, leukopenia/lymphopenia, and/or thrombocytopenia), neurologic abnormalities (seizures and psychoses), and vasculitis. Alleles at this locus were not associated with SLE itself. However, the distribution of the IL1RN allele frequencies in SLE patients with neurologic abnormalities differed from SLE patients with none ($p=0.021$ by Fisher's Exact Test). The distribution of IL1RN allele frequencies in SLE patients with hematologic abnormalities also differed from SLE patients with none ($p=0.001$), which was significant after a correction for multiple testing. In particular, the IL1RN1 allele frequency was increased in those with hematologic abnormalities (0.74 vs. 0.46, $p<0.001$). SLE patients with hematologic abnormalities had a significantly increased IL1RN*1 homozygote frequency compared to those patients with none (0.50 vs. 0.12, $p=0.001$). Stratification of SLE clinical phenotypes has identified a novel association of the IL1RN*1 allele with hematologic abnormalities, suggesting that the IL1RN gene functions as an SLE disease-modifier in this North-American Caucasian SLE cohort.

Program Nr: 2278 from the 1999 ASHG Annual Meeting

Cardiovascular risk factors in stroke cases, stroke family history positive, and stroke family history negative siblings: The HyperGEN Study. *J.B. Wilk[1], R.C. Ellison[1], D.K. Arnett[2], S.C. Hunt[3], S.S. Rich[4], Y. Hong[5], R.H. Myers[1]*. 1) Boston University School of Medicine, Boston, MA; 2) University of Minnesota School of Public Health, Minneapolis, MN; 3) University of Utah, Salt Lake City, UT; 4) Wake Forest University School of Medicine, Winston-Salem, NC; 5) Washington University, St. Louis, MO.

The goal of this study was to examine the differences in cardiovascular risk factors among three groups defined by their personal and family history of stroke. The participants were part of the HyperGEN study, a cohort of sibships ascertained for two or more hypertensive siblings, supported as part of the NHLBI Family Blood Pressure Program. The three groups compared were 1) (FamHx-) no family history and no diagnosis of stroke or transient ischemic attack (TIA); 2) (FamHx+) a family history of stroke or TIA, but no diagnosis of stroke or TIA; and 3) (stroke) any diagnosis of stroke or TIA. Mean levels of total cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides (TG), glucose, and uric acid were calculated for the three groups stratified on gender. Adjusted group means were also calculated separately for men and women to account for correlated observations between siblings and the effect of age, BMI (kg/m^2), alcohol use (yes/no), and physical activity (per week). Adjusted TG levels were significantly different in the three groups for both men and women. TGs increased from the lowest levels in the FamHx- group (men: 150 mg/dl, women: 131 mg/dl), intermediate levels associated with FamHx+ (166 mg/dl, 147 mg/dl), and the greatest levels associated with stroke (177 mg/dl, 158 mg/dl). For mean total cholesterol and glucose, the stroke group had significantly higher levels, and the mean levels in the FamHx- and FamHx+ groups were not significantly different. The stroke group had significantly lower adjusted mean HDL cholesterol levels, and HDL levels in the FamHx- and FamHx+ groups were not significantly different. Mean adjusted uric acid levels did not vary significantly across the three groups. These results suggest that a family history of stroke is associated with elevated TG levels in families with hypertensive siblings.

Program Nr: 2279 from the 1999 ASHG Annual Meeting

Multilocus SNP disequilibrium is associated with hypertension in a Ghanaian population. *S.M. Williams[1], J.H. Addy[2], J.A. Phillps III[3], M. Murray[1], L.-J. Wong[4], P.A. Jose[4], R.A. Felder[5]*. 1) Meharry Med Col, Nashville, TN; 2) Univ Ghana Med Sch; 3) Vanderbilt Univ Sch Med; 4) Georgetown Univ; 5) Univ Virginia.

Hypertension (HT) has been associated with SNPs or other mutations in components of the renin-angiotensin system (e.g., ACE, angiotensin-I-converting enzyme, AGT, angiotensinogen and AT1R, angiotensin II type I receptor) in separate studies of various populations. We hypothesized that multiple, non-allelic interactions at these loci might show stronger associations with HT than do separate alleles. A total of 126 HT and 51 non HT Ghanaian subjects were ascertained, phenotyped and their DNAs genotyped for the ACE ins/del, AGT T174M and M235T and AT1R A1166C alleles. Multilocus Hardy-Weinberg (H-W) analyses were performed to detect genetic associations between 2-4 of these loci. The ACE gene ins/del and the AT1R alleles were in H-W disequilibrium in the HT but not the non HT class ($p=0.01$ and 0.04, respectively). ACE genotypes showed an excess of heterozygotes ($f=-0.22$), while AT1R genotypes had a deficiency of heterozygotes ($f=0.39$) in the HT class. The AGT alleles showed no differences individually. However, significant multilocus interactions were observed in HTs in 2/11 multilocus comparisons, both involving AGT alleles: M235T and ACE ($p=0.02$) and T174M and AT1R ($p=0.02$). No significant differences were detected between the HT and non HT classes in allele or genotype frequencies at any locus and no significant deviations from H-W equilibrium were observed in the non HT class. Three SNPs at an additional, unreported locus exhibited even stronger disequilibrium. In summary: 17 of 120 (14%) possible multilocus comparisons across all 7 sites showed significant interactions ($p<0.05$) and 8 other comparisons had $0.05<p<0.10$ in the HT class, but no interactions with $p<0.24$ were observed in the non HT class. Our data indicate that a large number of multilocus comparisons showed significant linkage disequilibrium in the HT but not in the non HT classes. We infer that non-allelic interactions may be associated with HT and that multilocus SNP disequilibrium analysis may be useful in studies of the cause(s) of HT and other complex human diseases.

Program Nr: 2280 from the 1999 ASHG Annual Meeting

Population genetic inferences from nucleotide-sequence variation in human a **chain of the interleukin-4 receptor (*IL4RA*).** *X. Wu[1,2], A. Di Rienzo[1], M.S. McPeek[2], C. Ober[1].* 1) Department of Human Genetics, The University of Chicago, Chicago, IL; 2) Department of Statistics, The University of Chicago, Chicago, IL.

Variants in the *IL4RA* gene have been associated with asthma and allergy in ethnically diverse populations, including inbred Hutterites, outbred Caucasians, African Americans, Hispanic and Japanese. Here we report the initial results of a population genetic study of *IL4RA* variants in healthy individuals (72 chromosomes) from three ethnic groups: African Americans (24), Caucasians (24), and East Asians (24). A 3-kb region, including part of intron 8, entire exon 9, and the 3'UTR was sequenced. The sequence has a total of 23 polymorphic sites, including two new nonsynonymous substitutions, yielding a nucleotide diversity of 0.0018. Tajima's D was calculated to determine if the variants in this region are selectively neutral. The overall Tajima's D (0.42107) is not significant ($P > 0.1$), however D is positive (1.03352) for African Americans but negative for Caucasians (-0.50695) and East Asians (-0.73624), perhaps reflecting the different selective pressures in these populations. Comparisons between human and chimp yielded a total of 25 fixed differences in this region. The HKA Test was used to test for homogeneity of the evolutionary forces acting on different portion of the sequenced region. The result was not significant ($P = 0.7$), indicating that the evolution of the sequences in the intron, exon 9, and 3'UTR does not differ measurably. To further test the neutral hypothesis, we compared the divergence and polymorphism levels of nonsynonymous versus synonymous substitutions using the McDonald-Kreitman Test. There were 9 nonsynonymous and 4 synonymous sites in exon 9 in the human samples, whereas there were 8 nonsynonymous and 6 synonymous sites between human and chimpanzee (Fisher's exact test, $P = 0.69$). Although the test is not significant based on this data set, the high ratio of nonsynonymous to synonymous changes (2.25) may suggest that natural selection operated on this gene. Furthermore, this study further documents the presence of extensive variation in genes associated with common diseases. (Supported by NIH grants HL 49596, HL 56399 and NSF grant GIG DMS 97-09696).

Program Nr: 2281 from the 1999 ASHG Annual Meeting

Genetic Relationships of 15 Southeast Asian populations: mtDNA evidence. *C. Xiao[1,2], B. Su[2], M. Seielstad[3], E. Kangwanpong[4], H. Xiao[1], B. Wen[1], L. Jin[2].* 1) Department of Biology, Yunnan University, Kunming, Yunnan, China; 2) Human Genetics Center, University of Texas - Houston, Houston, TX; 3) Program for Population Genetics, Harvard School of Public Health, Boston, MA; 4) Department of Biology, Chiang Mai University, Chiang Mai, Thailand.

Sino-Tibetan, Hmong-Mien, and Austro-Asiatic are the major language families spoken in mainland Southeast Asia. In this study, we sampled 15 populations (6 Sino-Tibetan, 4 Hmong-Mien, and 5 Austro-Asiatic, 363 individuals in total) from China, Thailand and Vietnam in order to investigate the genetic relationships of those populations. Our results showed that the frequencies of mitochondrial DNA 9bp deletions vary drastically among different populations, ranging from 0 to 41.5%, and populations in the same language family tend to share similar 9bp-del frequencies, implying the concordance between the linguistic and genetic relationship in this area. In addition, we also sequenced the two highly variable segments (HVS-I and HVS-II) in the control region of individuals with the 9-bp deletion (70 samples). The so-called Polynesian motif (16217C, 16247G and 16261T), which was found exclusively in Polynesian populations, was not found in our samples. However, the intermediate ancestral haplotype (16217C, 16247A and 16261T) of the Polynesian motif were found in several populations, especially in Yao populations residing both in mainland China and in Thailand (23.1% in average). This observation makes Southeast Asia another possible homeland of the origin of pro-Polynesian people and therefore challenges the validity of the Taiwan homeland hypothesis on Polynesian origin.

Program Nr: 2282 from the 1999 ASHG Annual Meeting

High frequency of the FMR-1 INV10+14c/t polymorphism in Asians, and its association with the Fragile X Syndrome in Caucasians. *B. Xu[1,2], J.M. Schoof[2], N.E. Buroker[1], C.R. Scott[1,2], S.-H. Chen[1,2]*. 1) Department of Pediatrics, University of Washington, Seattle, WA; 2) Children's Hospital & Regional Medical Center, Seattle, WA.

Expansion of the CGG repeats in the FMR 1 gene is responsible for the fragile X syndrome. We have investigated an FMR I intragenic polymorphic site (IVS10+14c/t) in Asian, Caucasian and African-American populations in Seattle. A pair of primers (IVS10F: 5'-AGAAGAGGTATGTTACAGCG and IVS10R: 5'-ACTGCATTAGAGGACAGAGA) were synthesized and used for amplifying a fragment of DNA (187 bps) of intron 10 to target a BstU I (5'CGCG) recognition site. The PCR product of IVS10+14c was digested by the enzyme and generated two fragments of 167 and 20 bps. The enzyme did not digest IVS10+14t. We genotyped a total of 779 normal chromosomes for the polymorphism in 312 Asians, 208 Caucasians, and 159 African-Americans. The frequency of IVS10+14t in the Asians was 63.78%, higher than the frequency found in Caucasian (9.13%) and African-American (10.69%) samples. The high frequency of the polymorphism in the Asian population indicates this site is a useful marker for genetic analysis; but contrary to what has been suggested by others, it is unlikely to be a determining factor for fragile X syndrome in Asians. Analysis of the IVS10+14 polymorphic site in 50 Caucasian fragile X chromosomes showed that 4 were IVS10+14t. The chi-square test (x2) for these data was 0.13, with a p-value of >>0.05. This value was not different from the one predicted by random association in the Caucasian population. By contrast, tests of two other closely linked markers (DXS548 and FRAXAC1) showed that the fragile X chromosomes were associated with specific haplotypes.

Program Nr: 2283 from the 1999 ASHG Annual Meeting

Association of a polymorphism in the gene for MMP13 with atherosclerosis. *S. Yoon[1], G. Tromp[1], J. Olson[3], G. Malcom[4], J. Strong[4], H. Kuivaniemi[1,2].* 1) Center for Molecular Medicine and Genetics,Wayne State University School of Medicine, Detroit, MI; 2) Department of Surgery, Wayne State University School of Medicine, Detroit, MI; 3) Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, Ohio; 4) Department of Pathology, Louisiana State University Medical Center, New Orleans, LA.

Atherosclerosis causes more than 700,000 deaths per year from myocardial infarction and stroke in the USA. It is therefore the leading cause of death, accounting for 50% of all mortality. Atherosclerosis progresses through three stages: fatty streak, fibrous plaque, and complex lesion. Transition from fatty streak to other stages involves an inflammatory fibro-proliferative response that is accompanied by tissue remodeling. Several members of the matrix metalloproteinase (MMP) family, major extracellular matrix degrading enzymes, are highly expressed in atherosclerotic lesions. We identified a polymorphism of either 11 or 12 As at nt -291 in the MMP13 promoter. It is located upstream of an AP-1 binding site and spans sequences with similarity to consensus sequences for nuclear factors HFH-2 and Hb. We tested for association between the polymorphism and atherosclerosis in a subsample of the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) collection because of the unbiased collection and thorough pathological evaluation of atherosclerosis. The PDAY study was a multicenter autopsy study that examined risk factors for coronary artery disease, including smoking, hypertension, diabetes, and lipid profiles. DNA for genotyping was isolated from frozen liver specimens. We analyzed the MMP13 genotypes from 193 individuals with fibrous plaque and 268 without. Our logistic regression model included age, sex, race, and their interactions and yielded a p-value of 0.18, which was within our liberal criterion for a first stage of screening. Subgroup analyses, controlling for age, showed that the effect is almost exclusively due to black males, p-value < 0.01. Supported by a grant from American Heart Association-MI affiliate.

Program Nr: 2284 from the 1999 ASHG Annual Meeting

Statistical Methods for Examining Association Between Recombination and Non-disjunction. *K. Yu*[1], *E. Feingold*[2]. 1) Biostatistics, University of Pittsburgh, Pittsburgh, PA; 2) Human Genetics, University of Pittsburgh, Pittsburgh, PA.

Recent studies have found an association between altered recombination and meiotic non-disjunction leading to trisomy. Attempts to better understand this association have included using recombination data to estimate frequencies of various patterns of exchange on the tetrad, in order to see which particular exchange patterns are associated with non-disjunction. These studies have been somewhat hampered by limitations in the available statistical methods. We have developed improved statistical methods for this type of analysis based on the E-M algorithm and the bootstrap. Our methods allow better parameter estimation, including confidence intervals, and also allow statistical hypothesis testing. We have applied our methods to several published datasets, and compared our results with those in the original papers. For the most part the conclusions reached by previous methods stand up under more rigorous statistical examination, but there are some exceptions.

Program Nr: 2285 from the 1999 ASHG Annual Meeting

Molecular studies in a sample of Iranian phenylketonuria patients. *N. Zarrin-Khameh, S.M. Hosseini-Mazinani, J. Koochmeshgi, N. Hosein Pur.* Molecular Genetics, National Research Center for Genetic Engineering and Biotechnology, Tehran, Iran.

Phenylketonuria (PKU) is the most common error of amino acid metabolism. This autosomal recessive disorder is caused by a large variety of mutations in the phenylalanine hydroxylase (PAH) gene. To date, more than 300 different mutations in the PAH gene have been identified in patients from various ethnic groups. These mutations exhibit a high degree of association with specific RFLP and VNTR haplotypes in different populations. The study of PAH mutations in diverse populations contributes to our knowledge about population, history, and genetic variation in this locus. In a preliminary study of the PAH mutations in Iran , 10 unrelated Iranian classical PKU patients were screened for 5 known mutations: R261Q, R261X, R408Q, R408W, and IVS10nt546. The R261X mutation was observed in one patient and the IVS10nt546 mutation in two patients.All three were homozygous for the mutations. The R261X mutation involves a CpG doublet and has been found in Caucasians and East Asians but in association with different haplotypes. IVS10nt546, a splice junction mutation strongly associated with RFLP haplotype 6, is the most prevalent mutation in the PAH gene causing phenylketonuria in Mediterranean populations. It accounts for about 40% of mutant alleles in Iran's neighboring country, Turkey. Various and alternative hypotheses have been proposed concerning the geographical origin and pattern of diffusion of this mutation in this region of the world. Continued mutation screening and haplotyping in Iran will contribute to resolving this and other problems in the population genetics of phenylketonuria.

Program Nr: 2286 from the 1999 ASHG Annual Meeting

Effects of Cancer Chemotherapy on Frequencies of Minisatellite Mutations in Human Sperm. *N. Zheng[1,2], D.G. Monckton[1], G. Wilson[1], L. Ramagli[1], M.J. Siciliano[1], M.L. Meistrich[1].* 1) M.D. Anderson Cancer Center, Houston, TX; 2) Univ. Texas School of Public Health, Houston, TX.

Minisatellite repeat number changes have been proposed to be sensitive biomarkers for mutagenic effects of radiation and genotoxic chemicals in the human germ line. To test this hypothesis, we analyzed the frequencies of length change mutations at the MS205 locus in human sperm using small-pool PCR, with which we can screen 8000 sperm per sample and detect mutations in individual sperm. Sperm were obtained from patients before, during, and after cancer chemotherapy. Previously we showed that the mutation frequency after chemotherapy with, NOVP, which lacks alkylating agents, was the same as before treatment indicating no induction of mutations in stem spermatogonia. In the present study we measured the frequencies of mutations induced in primary spermatocytes by collecting sperm during chemotherapy. The results from three Hodgkin's disease patients treated with either NOVP or ABVD, another regimen that lacks alkylating agents with high testicular toxicity, indicated that repeat number mutation frequencies at MS205 during chemotherapy were identical to baseline values (before or after treatment). To analyze the effects of more highly mutagenic agents, sperm were obtained from three patients 9 to 15 years after MOPP or CVPP-ABDIC chemotherapy, which contain strong alkylating agents. The MS205 mutation frequencies in the two patients who received CVPP-ABDIC (3 to 4 cycles of each) were identical before and after chemotherapy. However the patient receiving 6 cycles of MOPP chemotherapy had a significantly higher repeat number mutation frequency of 1.14% at 15 years after treatment, compared to 0.79% before chemotherapy. Thus very high doses of alkylating agents can induce a significant increase in repeat number mutation frequencies at minisatellite loci but the increase at MS205 was not very large. From these data we conclude that either MS205 is not a very sensitive locus, or that repeat number changes at minisatellite loci are not sensitive indicators for detecting mutagenic changes, or that chemotherapy does not induce many DNA sequence mutations in germ cells.

Program Nr: 2287 from the 1999 ASHG Annual Meeting

Candidate Gene Loci for Endogenous Sex Steroid Hormones. *J.M. Zmuda, J.A. Cauley, L.H. Kuller, M.M. Johns, X. Zhang, M. Lee, R.E. Ferrell.* University of Pittsburgh, Pittsburgh, PA.

Endogenous sex steroid hormones may have a prominent role in the etiology of several age-related diseases in men including atherosclerosis, osteoporosis, and prostate cancer. A familial influence on sex steroid hormone levels has been demonstrated, but the specific genes and allelic variants contributing to their interindividual variation have not been defined. Thus, we quantified the association between allelic variation at four sex steroid metabolism genes and fasting levels of bioavailable testosterone (BT) and estradiol (BE) in 279 community-dwelling men aged 58-91 years who were not taking drugs known to affect sex steroid hormone metabolism. Unlinked marker loci at genes encoding the androgen receptor (*AR*; CAG repeat in exon 1), estrogen receptor alpha (*ESRA*; A®G in intron 1), cytochrome P450c17a (*CYP17*; T®C at position -34 bp), and aromatase (*CYP19*; Arg264Cys) were studied. Observed genotype frequencies did not deviate significantly from Hardy-Weinberg expectations. Hormone levels were adjusted for age and body mass index using multiple regression. Mean BT was 123±45 ng/dl and mean BE was 14.1±5.1 pg/ml. BT increased by 5.4% per copy of the *CYP17*C* and *ESRA*A* alleles ($p<.05$ for both), and by 1.0% per 6 CAG repeats in *AR* ($p=.01$). BT was 21.0% lower in carriers than non-carriers of the Arg264Cys mutation in *CYP19* ($p=.005$). BE increased by 1.1% per 6 CAG repeats in *AR* ($p=.01$) and was 18.5% lower in carriers of the Arg264Cys mutation in *CYP19* ($p=.03$). Candidate gene loci collectively explained 6.2% of the variance in BT and 3.8% of the variance in BE levels. We conclude that marker alleles at key sex steroid metabolic loci are significantly associated with circulating androgen and estrogen levels in older men. Additional studies are needed to determine the contributions of these marker loci to the risk of androgen and estrogen dependent disease.

Program Nr: 2288 from the 1999 ASHG Annual Meeting

Interpretation of molecular diagnostic testing in facioscapulohumeral muscular dystrophy (FSHD). *P.W. Lunt[1], A. Stephenson[2], L. Middleton[2], L.A. Tyfield[2].* 1) Clin Genet, Inst Child Hlth, Bristol Childrens Hosp; 2) Mol Genet, Southmead Hosp, Bristol, UK.

In offering a molecular diagnostic service for FSHD, DNA samples are received for exclusion of diagnosis as well as for confirmation. In the absence of any known affected gene product, diagnostic testing will for the foreseeable future be based on identifying a shortened array of 3.3kb repeats at 4q35 on one copy of chromosome 4. Distinction from the two copies of the homologous repeat array at 10q26 cannot yet be achieved with certainty owing to polymorphic exchange between these repeat arrays, thereby resulting in a lower sensitivity than specificity of testing, and lower confidence for exclusion than for confirmation of diagnosis. Use of pulse field gel electrophoresis (PFGE), or of a recently developed fragment-type dosage test, can determine the ratio of Bln-resistant : Bln-sensitive repeat arrays. Published data suggests 20% of the population are polymorphic for the exchange, with 19% of controls and 90% of FSHD having a fragment <32kb, and with hybrid repeats or deletion of p13E-11 hybridisation site found in 1-2% of cases. Based on this we present a table of likelihood for a D4F104S1 fragment to be associated with FSHD or not, according to its size, Bln-sensitivity, and ratio of Bln-resistant : Bln-sensitive fragments present. Meaningful interpretation of a molecular test result can then be achieved by combining this likelihood figure in Bayes calculation with an estimate of the prior likelihood for the subject to have FSHD. For this it is essential to have adequate clinical information, and close liaison with a clinician expert in FSHD.

For example: Bln-sensitive (E) fragments <32kb are predicted in 4.7%of FSHD and 19% of controls. With this a fragment ratio of 1 Bln-resistant(EB):2-3 Bln-sensitive (E) fragments gives a likelihood ratio (LR) for FSHD:control of 2:1, whereas a reverse fragment ratio of 2-3EB:1E would give LR of 1:60. A 2EB:2E fragment ratio would give a LR of 1:30. For a 32-38 kb Bln-resistant fragment, the same fragment ratios as above would give LRs of 80:1, 1:2 and 40:1 respectively.

Program Nr: 2289 from the 1999 ASHG Annual Meeting

Detection of a *de novo* mutation using the STR 44 marker in a Duchenne muscular dystrophy family. *M. Maheshwari, M. Kabra, S.S. Shastri, S. Arora, P.S.N. Menon.* Genetics Unit, Dept.Of Pediatrics, All India Institute Of Medical Sciences, New Delhi 110029, India.

Duchenne muscular dystrophy (DMD) is an X-linked recessive genetic disorder characterized by progressive muscle weakness. It affects about one in 3500 males. The female relatives of affected boys are at risk of carrying the disease allele. Since females have two X chromosomes, detection of the carrier status by deletion screening of mutant allele poses a problem due to the presence of one normal X chromosome which masks the effect of the other chromosome. Short tandem repeat polymorphic markers play an important role in carrier testing and detection of *de novo* mutations as they are inherited in Mendelian fashion. It is possible to identify the females at risk of being carriers for the disease allele by family linkage studies of DNA polymorphic sites within the dystrophin gene. We report here a case of *de novo* mutation with the use of dinucleotide repeat marker STR 44. A 25-year-old female consulted us to know her carrier status and the likelihood of development of DMD in her 3-year old son. Her brother was diagnosed as having DMD. Deletion screening by multiplex PCR showed deletion of exon 44 in her brother and not in her son. Carrier testing was performed by family linkage studies using STR markers. PCR products were run on an 8% polyacrylamide gel and silver stained. Analysis with STR marker 44 revealed deletion of this marker in her brother but not in her, her son and her mother. Heterozygocity at the STR 44 locus in her and her mother suggest a non-carrier status for them. Intron deletion in her brother and no deletion in her son suggest a *de novo* mutation in her brother and the improbability of development of DMD in her son.

Program Nr: 2290 from the 1999 ASHG Annual Meeting

Prevalence of factor V Leiden mutation in 241 Hellenic patients with homozygous b**-thalassemia.** *C.G. Makatsoris[1,3], T. Hatzis[2], H. Karageorgiou[3], F. Karabatsos[4], C. Fragodimitri[4], G. Makatsoris[1], C. Pantos[3], T. Antoniadi[1], H. Vrettou[5], M. Karagiorga-Lagana[4], M.B. Petersen[1], E. Economou-Petersen[5]*. 1) Department of Genetics & Molecular Biology, "Mitera" Maternity & Surgical Center, Athens, Greece; 2) Department of Hematology, "Mitera" Maternity & Surgical Center, Athens, Greece; 3) Department of Pharmacology, University of Athens Medical School, Athens, Greece; 4) Thallasemia Center, "Agia Sophia" Children's Hospital, Athens, Greece; 5) Thallasemia Treatment Unit, Drakpoulion Blood Bank Center, Athens, Greece.

Several clinical manifestations are associated with thromboembolic events which are frequently observed in b-thalassemic patients, such as deep venous thrombosis, arterial occlusions, pulmonary thromboembolism, etc. A key mechanism involved in the pathophysiology of thrombosis is a mutation localized in exon 10 of the factor V gene. Several studies have shown that there is a high incidence of this mutation among Hellenic individuals; one of the highest incidence rates in Europe. It's for these reasons that we conducted our study in 241 thalassemic major patients of Hellenic origin. The objective was to determine whether the 1691 G®A mutation of the factor V gene plays a role or has a synergistic effect for the development of thrombosis in b- thalassemia. In 241 patients, 3 were found to be heterozygotes for the FV Leiden mutation and one homozygote (carrier frequency 1.6; allele frequency 1.1). This is comparatively lower from the allele frequency found in healthy Hellenic blood donors which we have previously demonstrated to be 2.5%. An explanation could be that thallasemic patients having the FV Leiden mutation carry a heavy survival disadvantage and possibly have already deceased. This coincides with the hypothesis that in b-thalassemic patients the prevalence of Factor V Leiden is low due to negative selection.

Program Nr: 2291 from the 1999 ASHG Annual Meeting

Rapid mutational screening of the Menkes/Occipital horn syndrome locus. *P.E. McAndrew[1], L.A. Keppen[2], S.G. Kaler[1,3]*. 1) Res Ctr for Genetic Medicine, Children's Research Institute; Washington, DC; 2) Univ So Dakota; Sioux Falls, SD; 3) Clin Neurocardiology Section NINDS/NIH; Bethesda, MD.

Menkes disease (MD)is an X-linked recessive neurodegenerative disorder of copper (Cu) transport. MD patients are unable to absorb and utilize Cu, and most clinical features can be explained by malfunction of essential cuproenzymes. The MD gene encodes a copper-transporting P-type ATPase, and lack or dysfunction of this protein leads to death in early childhood for 95% of classical MD patients. Occipital horn syndrome (OHS), an allelic variant of MD, has a milder neurological presentation and is associated with connective tissue abnormalities.'

'Mutation analyses by several groups demonstrated that approximately 20% of MD patients have large deletions in the MD/OHS gene, while the majority have small insertions/deletions, nonsense, missense, or splice site mutations. OHS patients typically have mutations at non-consensus splice site positions and maintain some proper splicing. Our laboratory is interested in defining mutations in Menkes and OHS patients in order to (1) better understand the molecular basis of this disorder; (2) understand the structure/function relationships (3) correlate molecular data with response to copper histidine treatment;(4) provide information for families interested in carrier detection and prenatal diagnosis of this fatal disorder.'

'Each of the 23 Menkes/OHS exons and flanking sequence is PCR amplified from genomic DNA and used for heteroduplex analysis as an initial screen to identify novel mutations. A multiplex PCR reaction is also being developed as an efficient method to detect gene deletions. In 1 at-risk male infant born prematurely at 28 wks gestation, we detected a deletion of exons 7-19 and used long PCR to identify a '»'3 kb junction fragment in him and his obligate carrier mother. He began treatment with copper histidine injections on day 9 of life. At 8 mo of age (5 mo corrected for prematurity), he shows developmental delays, failure to thrive, and hypertension related to renal artery aneurysms.

Program Nr: 2292 from the 1999 ASHG Annual Meeting

Advances in Bone Marrow Transplant Monitoring. *L.K. McGlynn-Steele, L.S. Horrocks, Y. Yang, C.A. Ryan, P.N. Ray.* Dept Pediatrics & Lab Med, Hosp Sick Children, Toronto, ON, Canada M5G 1X8.

The monitoring of bone marrow transplant (BMT) or peripheral donor blood infusion (PDBI) is essential to ensuring that the donor cells have successfully replaced either cancerous cells or cells deficient for certain genetic components. Previously, the ratio of donor to recipient cells present in the post-transplant sample was determined by examining the sex chromosomes of recipient cells. However, this method is very labor intensive and only useful when the sex of the donor and recipient were different. Molecular genetics offers new methods for monitoring BMT or PDBI patients. This new methodology involves using the GenePrintTM Fluorescent STR DNA fingerprinting procedure. The sample requirements for molecular analysis include a sample from the recipient pre- and post-transplant, as well as a sample from the donor. A total of eight microsatellite autosomal markers are examined in a multiplex-PCR reaction, in addition to both X- and Y-specific marker analyses using the AMELX/Y locus. This analysis allows the detection of admixtures of DNA down to the level of ~10%. We have tested more than 150 cases in a 1 year period. The advantages of this methodology over conventional cytogenetics include: 1) 3-fold less workload 2) the majority of BMT cases are informative 3) as little as 1ng DNA is required as starting material and 4) results can be obtained in time. Our results have provided invaluable information to clinicians by determining the status of the transplant and hence have had a positive impact on patient management. Examples from 4 BMT-monitoring cases will be presented here. These cases illustrate the importance of this method in the careful monitoring of the cell transition and long-term clinical prognosis.

Program Nr: 2293 from the 1999 ASHG Annual Meeting

Connexin-26 (Cx-26) gene mutation detection using Guthrie cards from subsequently identified deaf children.

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Prelingual sensorineural hearing loss (SNHL) affects approximately 1 in 500 children. Inherited deafness has been demonstrated to account for 50-60% of all SNHL. About 70% of inherited deafness is non-syndromic, the majority having autosomal recessive (AR) inheritance. About 50-80% of all AR deafness may be due to mutations in the Cx-26 gene. The 35delG mutation in this gene accounts for about 90% of all mutations characterized thus far.

Rhode Island established universal neonatal hearing screening in 1993. About 32 children per year are identified, about half with bilateral deafness needing amplification by 2 months of age. The aim of this study was to determine the efficacy and utility of Cx-26 gene analysis from DNA isolated from Guthrie cards and to precisely define a major cause of prelingual non-syndromic SNHL in those children requiring amplification and detected in this program.

50 deaf children were identified as non-syndromic in the last 5 years with no known cause for their hearing loss. 49 Guthrie cards were obtained from the RI State Lab following informed consent. 3 children were identified as 35delG homozygotes. 3 children were identified as 35delG heterozygotes. 38 children tested negative for the common 35delG mutation.There was no amplification of DNA or uninterpretable results using 5 Guthrie cards. 12% of children were identified with at least one Cx-26 mutation. The Cx-26 gene of the 3 heterozygous children is being sequenced.

Cx-26 analysis is an important test that identifies a major cause of prelingual non-syndromic deafness. Linkage to newborn screening programs will be important in identifying this major cause of deafness, facilitating genetic counseling and carrier detection in these families. Failures with Guthrie cards may make use of other methods preferable.

Program Nr: 2294 from the 1999 ASHG Annual Meeting

A brazilian study of FRAXE mutation in mentally retarded patients using OxE18 probe. *M.V. Mulatinho[1], J.C. Llerena[2], M.M.G. Pimentel[1]*. 1) Cell and Genetic Biology, UERJ, Rio de Janeiro, Brazil; 2) Instituto Fernandes Figueira, IFF/FIOCRUZ, Rio de Janeiro Brazil.

The expansion of trinucleotide repeats within the transcribed regions of genes has been associated to several genetics human disorders. Besides FRAXA, a second fragile site, FRAXE, that lies ~ 600 kb distal to the fragile X syndrome (FRAXA), has been characterized at Xq27.8, which is associated with a non-syndromic form of mental retardation (MR). The FRAXE prevalence is calculated as 1:50000 livebirths. Since 1996, the Human Genetic Service of State University of Rio de Janeiro, Brazil (UERJ), have been searching for FRAXA and FRAXE mutations by molecular technique in MR patients. The screening for FMR1 gene, has been based in standards protocols using EcoRI digests on Southern blots and hybridized with StB12.3 probe. For FRAXE mutation we have been using a molecular protocol for OxE18 probe, in the DNA samples digested with EcoRI . In this sample, 144 negative FRAXA patients (52 sporadic cases, 7 familial cases and 85 institutionalized patients) were tested by this methodology and no FRAXE mutation was detected. In our investigation 40 ng of OxE18 probe labeled with [a32P]dCTP 3000 Ci/mmol (Amersham)by random priming was left hybridizing overnight in 50 % of formamide in the same blots used for FRAXA screening, followed by 3xSSC, 1x SSC, 0,5x SSC washes at 42C. The autoradiograms showed a normal pattern of 2,5 kb for males and 2,5/8,5 kb for females showing the practical use to test FRAXA and FRAXE in a large amount of patients. The OxE18 is an alternative probe to investigate FRAXE mutation as it offers several advantages since it can be used to detect FRAXE locus expansion in the same Eco RI blots used for FRAXA screenings. This work was supported by CNPq, CAPES, FAPERJ, CEPUERJ.

Program Nr: 2295 from the 1999 ASHG Annual Meeting

California's Legislative Response to Genetic Discrimination. *A.M. Musial[1], J.A. Kopczynski[2]*. 1) Perinatal Practice Management, Walnut Creek, CA; 2) San Francisco, CA.

Recent advances in genetic research have increased both the availability and number of genetic tests for the health care consumer. A negative consequence of these promising technological advances, however, is that asymptomatic individuals identified as having disease genes are experiencing discrimination by their employers, health insurers, and health maintenance organizations based solely on their predisposition for such diseases. As a result of such acts of discrimination, many patients are compromising their health care by declining genetic testing that may help guide the patient and physician in choosing appropriate surveillance and medical interventions. Beginning in 1994 and continuing through 1998, the California Legislature responded by passing vanguard pieces of legislation prohibiting discrimination on the basis of one's genetic characteristics. This series of California laws specifically targets discrimination by employers, insurers, health plans and HMOs, and attempts to fill significant voids left by comparable federal genetic discrimination laws. As incidents of genetic discrimination become increasingly more prevalent in a society on the verge of fully mapping the human genome, California citizens now have legal recourse available to protect against these types of abuses by their employers, insurers, and HMOs. However, the existence, specifics and limitations of these laws are relatively unknown to the public. By applying this relatively recent body of California law to several hypothetical factual scenarios of discrimination based on recent case studies, California health care providers and citizens will be made aware of a patient's legal rights and remedies in this new era of discrimination on the basis of one's genetic information.

Program Nr: 2296 from the 1999 ASHG Annual Meeting

Trends in predictive and prenatal testing for Huntington disease, 1993-1999. *M.A. Nance[1], R.H. Myers[2], US Huntington Disease Genetic Testing Group[3]*. 1) Park Nicollet Clinic, St Louis Park, MN; 2) Boston University School of Medicine, Boston, MA; 3) Multiple Institutions, US.

Direct analysis of the CAG repeat sequence in the huntingtin gene has been used for predictive and prenatal testing of Huntington disease (HD) since 1993. To monitor the use of, and problems associated with, this gene test, the United States HD Genetic Testing Group, a consortium of clinical centers and laboratories providing the test, was formed in 1995. This group surveys its members yearly to determine how many tests have been performed, to identify unusual cases, and to obtain detailed information about any social complications of testing, such as employment or insurance discrimination, or litigation related to predictive testing.

The 1999 survey obtained information from 31 clinical centers or clinical centers with affiliated laboratories, and 2 commercial laboratories. Responding clinical centers had an average of 4.5 years of experience with testing, and had performed 1353 predictive tests, of which 57.9% were normal (no CAG repeat expansion), 39.9% showed an expansion (CAG repeat number >39), and 4.1% yielded a result in the incomplete penetrance range (36-39 CAG repeats). The 31 centers performed between 220 and 300 tests each year between 1994-1998. Only 20 prenatal tests have been performed by these centers since 1993. Social complications known to the test centers will be reviewed. Anonymous testing was performed by about one third of the centers in 1998.

The uptake of prenatal genetic testing for HD is currently very low. This study included about half of the US testing centers. Assuming that the other centers are performing a similar number of tests and that some tests are taking place outside of established centers, about 500-600 predictive tests are performed in the US each year, representing about 0.5% of the estimated 120,000 at-risk individuals per year, or up to 1 percent of at-risk adults under age 60.

Program Nr: 2297 from the 1999 ASHG Annual Meeting

Analysis of both *TSC1* and *TSC2* for germline mutations in 126 unrelated patients with tuberous sclerosis. *Y. Niida[1], N. Lawrence-Smith[1], J. Lewis[1], R.L. Beauchamp[1], K. Sims[2], V. Ramesh[1], L. Ozelius[3].* 1) Molecular Neurogenetics Unit, Massachusetts General Hospital, Charlestown, MA; 2) Department of Neurology, Massachusetts General Hospital, Charlestown, MA; 3) Molecular Genetics, Albert Einstein College of Medicine, Bronx, NY.

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder characterized by the development of multiple hamartomas involving many organs. About two-thirds of the cases are sporadic and appear to represent new mutations. With the cloning of two causative genes, *TSC1* and *TSC2*, it is now possible to analyze both genes in TSC patients and identify germline mutations. Here we report the mutational analysis of the entire coding region of both *TSC1* and *TSC2* genes in 126 unrelated TSC patient, including 40 familial and 86 sporadic cases, by single stranded conformational polymorphism (SSCP) analysis followed by direct sequencing. Mutations were identified in a total of 74 (59%) cases, including 16 *TSC1* mutations (5 sporadic and 11 familial cases) and 58 *TSC2* mutations (42 sporadic and 16 familial cases). Overall, significantly more *TSC2* mutations were found in our population ($c2 = 12.963$, $p = 0.00032$), with a relatively equal distribution of mutations between *TSC1* and *TSC2* among the familial cases, but a marked underrepresentation of *TSC1* mutations among the sporadic cases ($p = 0.0035$, Fisher's exact test). All *TSC1* mutations were predicted to be protein truncating. However in *TSC2*, 13 missense mutations were found, five clustering in the GAP-related domain and three others occurring in exon 16. Upon comparison of clinical manifestations, including the incidence of mental retardation, we could not find any observable differences between TSC1 and TSC2 patients. Our data helps define the distribution and spectrum of mutations associated with the TSC loci and will be useful for both understanding the function of these genes as well as genetic counseling in patients with the disease.

Program Nr: 2298 from the 1999 ASHG Annual Meeting

Use of fast high pressure liquid chromatography for analysis of thrombophilic mutations. *S.B. Parks[1], E. Gerber[2], R. Hatch[2], R. Foss[2], R.A. Luhm[3], M.J. Hessner[3], D.B. Bellissimo[3], R. Press[1], B. Popovich[1]*. 1) Oregon Health Sci Univ, Portland, OR; 2) Varian Inc, Walnut Cove, CA; 3) Blood Center of Southeastern Wisconsin, Milwaukee, WI.

In DNA diagnostic labs, analysis of thrombophilic mutations constitutes a growing percentage of the tests conducted. In an effort to increase clinical laboratory efficiency, we have evaluated the application of semi-automated fast high pressure liquid chromatography (F-HPLC) as an alternative to gel electrophoresis for analysis of PCR-amplified DNA fragments. In a blinded study, we have used the standard *Mnl* I RFLP followed by F-HPLC to analyze 112 patient samples for the factor V Leiden mutation, and have found all F-HPLC genotypes to be consistent with those generated by electrophoresis. Analyzing each sample for approximately 7 minutes (after PCR and digestion), we were able to visualize amplicon fragments ranging from 67 to 587 bp. Two notable advantages of F-HPLC are the automated analysis and the resulting electronic data. Once initiated, no further supervision is required during the analysis. Linking clinical laboratory computer systems with the electronic record generated for each sample can reduce the risks of transcription and interpretation errors. Our experience indicates that F-HPLC generates accurate, consistent, and reliable results, and can serve as a replacement for the gel electrophoresis of most PCR RFLP assays in the clinical setting.

We are currently conducting a blinded study analyzing 100 patient samples for the thrombophilic factor V Leiden, prothrombin G20210A, and MTHFR C677T mutations, using a multiplex ASO PCR in conjunction with F-HPLC. The ASO PCR detects all three mutations simultaneously without the need for restriction digestion. Coupling this technique with F-HPLC will provide a fast, efficient, semi-automated means of simultaneously detecting these three primary thrombophilic mutations in a clinical diagnostic setting. In conclusion, we believe that F-HPLC represents a promising new approach to molecular diagnostics by providing a semi-automated method of DNA analysis for RFLP-based or ASO PCR based protocols.

Program Nr: 2299 from the 1999 ASHG Annual Meeting

Measuring distress in individuals presenting for cancer genetic testing: A single interview question versus standard measures. *A.F. Patenaude[1,2], M. Dorval[1], L. DiGianni[1], K. Kalkbrenner[1], K.A. Schneider[1], S.A. Kieffer[1], J.E. Garber[1,2]*. 1) Dana Farber Cancer Inst, Boston, MA; 2) Harvard Medical School, Boston, MA.

Measurement of distress in persons presenting for genetic testing is important for clinical safety and evaluation of psychological outcomes. Depressed individuals may be at increased risk for adverse outcomes. Research programs have utilized interviews and standard psychological measures. In clinical settings, however, limits on time and personnel restrict assessment options.

We compare use of a single question assessing depressive affect with standard depression measures in 2 cancer genetics counseling programs. For 31 LFS family members seeking *p53* testing, the single question, "During the past month, have you experienced any periods of depression or consistent unhappiness?" correctly identified all subjects with T-scores of ≥63 on the Symptom Checklist-90R (sensitivity 100%), and identified 75% of those not meeting criteria for depression (specificity). Among 47*BRCA1*testing program participants, specificity of the single item remained high (85%), but sensitivity was lower (67%) when compared to Depression T-scores (≥63)on the Brief Symptom Inventory, a short version of the SCL-90R. Implications for affective assessment in clinical and research programs of cancer genetic testing are discussed.

Program Nr: 2300 from the 1999 ASHG Annual Meeting

Mutation detection for Epidermolysis Bullosa in a global population. *E.G. Pfendner[1,2], A. Nakano[1,2], K. Nielsen[1,2], L. Pulkkinen[1,2], J. Uitto[1,2].* 1) Dermatology and Cutaneous Biology; 2) DebRA Molecular Diagnostics Laboratory, Thomas Jefferson University Phila., PA.

Epidermolysis Bullosa (EB) is a group of heritable skin blistering disorders marked by extreme fragility of the skin and separation of epidermal layers within the cutaneous basement membrane zone either below the lamina densa (dystrophic EB, DEB), within the lamina lucida (junctional EB, JEB) or within the basal keratinocytes (EB Simplex). Molecular diagnosis for DEB and JEB has been performed for a group of patients from an international referral base representing patients from Africa, Asia, Australia, Europe and the Americas. To date, a total of 266 samples have been submitted which meet the diagnostic criteria for analysis; among these cases 144 are DEB and 122 are JEB. Of the patients in the DEB category, 19 were known to have dominant and 125 recessive inheritance patterns, while all known JEB cases were recessive. Using heteroduplex analysis followed by nucleotide sequencing, the mutation detection rate was 64% for DEB and 80% for JEB. DEB mutations have been detected in the COL7A1 gene in five categories; splice junctions (19.6%), missense (4.5%), nonsense (16.9%), insertion/deletion (32.1%) and glycine substitutions (26.8%). Recurrent DEB mutations have been identified at 4 sites comprising 6.2% of the total population surveyed. JEB mutations have been identified in six different genes; LAMA3, LAMB3, LAMC2, a6 and b4 integrin (ITGA6 and ITGB4) and BPAG2 (COL17A1). JEB mutations were identified in splice junction (15.6%), missense (6.4%), nonsense (37.7%) and insertion/deletion (40.2%) categories. Recurrent LAMB3 mutations accounted for 54.0% of all LAMB3 mutations and 32.5% of all JEB mutations. R635X was the predominant LAMB3 mutation comprising 41.1% of all JEB mutations. These molecular analyses lead to prenatal diagnosis for 58 pregnancies, 39 DEB and 19 JEB. Linkage analysis was used for prenatal diagnosis in another 10 DEB families. In 65 pregnancies genotype was correctly predicted. Three pregnancies are ongoing. These results indicate that DNA based prenatal testing is expedient and reliable in predicting genotype in pregnancies at risk for recurrence of EB.ab.

Program Nr: 2301 from the 1999 ASHG Annual Meeting

High-Throughput Mutation Detection at the Australian Genome Research Facility. *K.F. Poetter[1], S. Foote[1], J. Barlow[1], A. Wirapati[1], G. Myers[1], R. Cotton[2].* 1) AGRF, Walter & Eliza Hall Institute, Parkville, VIC, AUSTRALIA; 2) Mutation Research Center, St. Vincent's Hospital, Fitzroy, VIC, AUSTRALIA cotton@ariel.its.unimelb.edu.au.

Genomic research is rapidly changing the scope as well as focus of genetic research into causes of inherited diseases. With the rapid increase in primary DNA sequence information, the direct comparison of test DNA to known sequence as a way to characterise mutations is a methodology in need of development. To this end, the Australian Genome Research Facility (AGRF) has developed high throughput (> 2000 samples per day) protocols for chemical cleavage of mismatch (CCM) mutation detection. This report describes the robotic controls for CCM as well as rates the method on speed, accuracy (both false negative and false positive rates) and cost. Two main technical advances of this work are the development of **solid phase supports** for the chemical modification and cleavage steps of CCM and the **computerized automated subtraction protocols** which allow quick and accurate allele calling. Proof of principle and proof of practice experiments using a set of 15 Brca1 patients whose genotypes were determined by other investigators were analyzed by both CCM and direct sequencing. We have found that CCM can identify mutations with a low false negative rate. DNA sequencing of all amplimers of all patients failed to identify mutations which were unexpected from CCM results.

Program Nr: 2302 from the 1999 ASHG Annual Meeting

SMA carrier testing: the Ohio State University experience. *T.W. Prior[1], D.W. Parsons[1], J.W. Heinz[1], A.C. Papp[1], R.W. Schafer[1], M.S. Sedra[1], P.J. Snyder[1], A.H.M. Burghes[2], P.E. McAndrew[1].* 1) Department of Pathology, Ohio State University, Columbus, OH; 2) Department of Neurology, Ohio State University, Columbus, OH.

Autosomal recessive spinal muscular atrophy (SMA) is caused by homozygous loss of the telomeric survival motor neuron gene (telSMN) in ~95% of SMA patients. A quantitative competitive PCR assay for the determination of telSMN copy number was developed in our laboratory. This test allows the accurate identification of SMA and has been offered to SMA families in which a homozygous telSMN deletion has been ascertained. We now report the results of the first two years of clinical SMA carrier testing at our institution.

The assay has been performed on 181 individuals, most frequently spouses of SMA carriers or patients (n=52) and aunts/uncles (n=48) or parents (n=44) of SMA patients. The test results for our patient population closely resemble those expected for an autosomal recessive disorder: 12 of 16 siblings (75%) of SMA patients and 20 of 48 (42%) aunts/uncles of patients were determined to be carriers. Two of 52 unrelated spouses of SMA carriers or patients were found to be carriers, which is not significantly different from the estimated SMA carrier frequency of 1/40 to 1/50 individuals. Consistent with a reported new mutation rate of 2%, 1 of 44 parents of SMA patients was revealed to possess two copies of telSMN.

These results demonstrate the presence of significant interest in SMA carrier testing, and confirm that this assay can reliably identify carriers of SMA as previously established. Furthermore, no asymptomatic individuals were found to be homozygously deleted for telSMN in our patient population, indicating that this phenomenon is a rare occurrence and does not affect carrier studies significantly. In families of SMA patients with homozygous loss of the telSMN gene (and not individuals with telSMN point mutations or other neuromuscular disorders), this test provides an accurate means of determining SMA carrier status and allows at-risk individuals to make informed family planning decisions.

Program Nr: 2303 from the 1999 ASHG Annual Meeting

Attitudes to genetic testing in childhood in England and Wales. *A.M. Procter, A.J. Clarke, P.S. Harper.* Institute of Medical Genetics, Uni Wales College of Medicine, Cardiff, U.K.

It is 5 years since the survey carried out by a Working Party of the UK Clinical Genetics Society contributed to its report entitled "The Genetic Testing of Children". Since that time, the debate has continued, particularly with regard to the desirability and organisation of such tests. As the range of tests available has increased, so has public awareness of genetic issues. This interest has been fostered by increased ease of access to medical and scientific information which has in turn generated new pressures upon individuals involved in genetic counseling. In collaboration with the Department of Health and the Advisory Committee on Genetic Testing, we have instituted a survey which aims to assess current opinion among the various groups of professionals involved in the genetic testing of children. Two thousand questionnaires have been circulated to clinicians and senior scientists in molecular and cytogenetic laboratories throughout England and Wales. Those returned (more than 700) have allowed us to gain some insight into current trends both in professional practice and in attitudes to the use of genetic tests in childhood.

Program Nr: 2304 from the 1999 ASHG Annual Meeting

Screening of the C282Y mutation in the HFE gene in Italy by Taqman technology. *G. Restagno[1], A.M. Gomez[1], L. Sbaiz[1], S. Bosso[1], C. Negro[1], P. Fortina[2], P. Gasparini[3], M. Cicilano[4], M. De Gobbi[4], C. Camaschella[4].* 1) Clinical Genetics, A. O. O.I.R.M.-S. ANNA, Torino, Italy; 2) Department of Pediatrics, The Children Hospital of Phildelphia PA; 3) Medical Genetics Service, IRCCS, San Giovanni Rotondo, Foggia, Italy; 4) Department of Clinical and Biological Sciences, University of Torino.

Hereditary Hemocromatosis (HH) is a common autosomal recessive disorder of iron metabolism. Homozigosity for a C282Y mutation in the Hemocromatosis gene (HFE) is the underlying defect in approximately 64-95% of patients with HH. Carrier frequency in Caucasians ranges between 3-13%. Gene frequency in a cohort of italian newborns using a new assay based on the use of two fluorescent dyes (a reporter and a quencher) both attached to the probe. Fluorescence is read with the ABI Prism 7700 Sequence detection System. The C282Y mutation was evaluated in 554 newborns. No newborns were homozygous for the C282Y; there were 18 C282Y heterozygotes which translates into a carrier frequency of 3,2%. In order to asses the presence of genotypes at risk other than C282Y homozygosity, the mutation H63D was investigated in 18 heterozygotes and 3 resulted compound heterozygotes (5,4/1000). However the risk of compound heterozygotes is rather low. The frequency of C282Y in Italy is lower than that described in Brittany, where the homozygote frequency was 5/1000 and heterozygote frequency was 12%. This may be explained because HH is considered as the most frequent disease in the population of Northern Europe origin; the origin of heterozygous newborns described in our work was mixed (North, Center and South of Italy) with the exception of 5 cases from Northern Italy. Since the current study is done blindy, we expect that the frequency of the C282Y mutation is not biased. The use of Guthrie spots coupled with the Taqman methodology proved to be semi-automatable, rapid and suitable for screenings of the general population.

Program Nr: 2305 from the 1999 ASHG Annual Meeting

Testing for an Inherited Susceptibility to Breast-Ovarian Cancer: Consequences One Year Later. *P.T. Rowley, S. Loader, J.C. Levenkron, C.G. Shields.* University of Rochester, Rochester, NY.

Concern has been expressed about the consequences of BRCA testing. Will women in whom a mutation is found be excessively worried? Will women in whom no mutation is found be inappropriately reassured and reduce surveillance?

We conducted a trial in which free genetic counseling and BRCA testing were offered to any woman in the care of a Monroe County physician who had two or more first- or second-degree relatives with breast or ovarian cancer. Of 140 women qualifying, 112 came for individual pretest education (averaging 90 min.), and, after hearing about risks and limitations as well as benefits, 98 chose to be tested. Of the women having cancer from 87 families actually tested, 13 had a deleterious BRCA1 or BRCA2 mutation and were given information about recommended surveillance and optional prophylactic surgery.

At 1 mo. and at 1 yr. after receiving their test result, patients were evaluated by structured interview and questionnaires. Knowledge of genetic risks for breast cancer was significantly greater at 1 mo. than before education ($p < .001$) and was well maintained at 1 yr. Breast Cancer Worry score did not significantly rise in any test result group, even in those found to have a mutation. At 1 yr., Breast Cancer Worry score was higher in women who perceived breast cancer as more serious, but not in women who perceived themselves as more susceptible. Scores for self-assessed breast cancer surveillance at 1 yr. did not differ among test result groups. Of the 87 women tested, 41 were very satisfied, 19 somewhat satisfied, and only 13 neutral or dissatisfied with the testing program. Compared to 1 mo., satisfaction at 1 yr. was increased in 40 cases, unchanged in 13 cases, and decreased in 9 cases.

Thus, at 1 yr., we observed that knowledge had been retained, worry about breast cancer had not risen even in those found to have a mutation, surveillance was satisfactory, and most were satisfied with the testing program. We attribute these favorable results in part to a significant investment in pretest education, generating realistic expectations among those choosing testing and permitting others to choose no testing.

Program Nr: 2306 from the 1999 ASHG Annual Meeting

Under utilization of genetics services in a poor urban population . *H.M. Saal[1], L. Hoechstetter[1], C. Hetteberg[2], E.K Schorry[1]*. 1) Div Human Genetics, Children's Hosp Medical Ctr, Cincinnati, OH; 2) College of Nursing, University of Cincinnati.

The Division of Human Genetics is the sole provider of genetics services in southwest Ohio, serving a population of 1.9 million. We successfully provide outreach services in 7 distant rural counties. In our own county, which is urban, we have identified a significantly under served poor population, which, for various reasons has had limited utilization of specialty services. Analysis of the utilization of genetic services revealed startling differences when comparing individuals from the wealthiest and poorest zip codes in our community. The median income for the wealthy zip code (WZP) was $43,375 compared to $8,824 for the poor zip code (PZP). The racial distribution for the WZP was 91% Caucasian and for the PZP 77% African American. During fiscal year 1997 there were 37 patient visits from the WZP compared to only 12 from the PZP, and only one patient had private insurance. For genetic encounters, individuals from combined wealthier communities were seen predominantly for outpatient visits (88%), whereas inpatient consultations accounted for almost 50% of genetics visits for persons from poor communities. In order to identify the reasons for the differences in provision of clinical genetics services to these poor communities, we have embarked on a project to study the barriers to genetics services in three urban primary care clinics. The study includes educational programs for primary care health providers, as well as assessing the role of case management in provision of genetics services. Since the initiation of the project, we have seen patients with a wide variety of conditions. Early data have identified a lack of understanding of the role of clinical genetics in the health care management of children with genetic disorders by both the lay public and primary healthcare providers along with an under-identification of individuals who would benefit from genetics services. From these data it is clear that we must address the modes of provision of genetics services to poor urban populations. Supported by Maternal and Child Health Bureau grant MCHG 98-07.

Program Nr: 2307 from the 1999 ASHG Annual Meeting

Economical analysis of mutation detection: DHPLC as contract services to enhance discovery in the Human Genome Variability. *M. Sanders[1], B. Lobos[2], R. Todd[2].* 1) Psychiatry, GeneShop Inc., Washington Univ, St Louis, MO; 2) Psychiatry Department, Washington University School of Medicine, St. Louis, MO.

A growing need to detect de novo sequence variants in newly discovered human genes presents challenges to the research community for rapid and accurate delivery of data. We analyze various mutation detection techniques and discuss DHPLC as a contract service available to the human genetics laboratories. DHPLC employs the formation of heteroduplexes between wild-type (reference) and mutated DNA, which are efficiently separated on the WAVE DNA Fragment Analysis System (Transgenomic Inc., San Jose, CA). GeneShop Inc. offers services for mutation/SNPs discovery, which are competitive in price and complementary to sequencing, thus reducing research costs in search of sequence variants. Temperatures, maintained by the WAVE oven, finely mediate partial melting (denaturation) of the heteroduplexes at mismatch sites. The melted domains in the heteroduplex molecular species cause them to be retained less strongly on the DNASep micropellicular matrix of the WAVE analytical cartridge than the double-stranded homoduplex molecules. We demonstrate the superiority of this approach to gel-based techniques and sequence-it-all projects. For additional info geneshop3@hotmail.com, Tel. 314 362 8650.

Program Nr: 2308 from the 1999 ASHG Annual Meeting

Molecular diagnosis of congenital deafness: novel mutations in the GJB2 gene. *L. Sbaiz, A.M. Gomez, C. Carbonara, S. Bosso, C. Negro, G. Restagno.* Clinical Pathology, A.O. O.I.R.M.-S.Anna, Torino, Torino, Italy.

The most common form of inherited and apparently sporadic congenital deafness is due to mutations in the connexin-26 gap junction protein B2 (GJB2) on human chromosome 13q11 (DFNB1). The 35delG frameshift mutation accounts for 60% of the DNFB1 alleles in the Mediterranean population, representing the most common mutation . We search for GJB2 mutations in congenital deaf and in potential carriers within their families using a direct sequencing approach.In the present study we report on the molecular analysis of the Cx26 gene in a group of 25 patients and 59 potential carriers. Analysis of the complete coding region of the GJB2 gene revealed seven different mutations: one frameshift (35 delG) three stop codons (E47X, W24X, and a novel mutation, E114X) and three aminoacid substitutions (R127H, the novel mutations Q80P and V153I). An additional novel mutation (V37I, aminoacid substitution) has been found in a three generation autosomal dominant family, with a form of late onset (45 years of age) sensorineuronal deafness: the mutation segregates in all the seven affected members. Nine patients (36%) were found to be 35delG homozygous, 2 patients were compound heterozygous E47X/35delG, and one E114X/35delG; four patients showed only one mutation and 9 (36%) showed no mutations. Severe deafness or hearing impairment is the most prevalent inherited sensory disorder, affecting about one in 1000 children. Although many cases of congenital deafness are tough to be recessively inherited, the nature of sporadic cases is unknown, which makes genetic counselling and the diagnosis of deafness difficult. Mutations in the GJB2 gene represent a major cause of congenital deafness; the characterization of 35delG and other mutations facilitates population screening and diagnosis and counselling in families with affected members.

Program Nr: 2309 from the 1999 ASHG Annual Meeting

Impact of neonatal screening and prenatal diagnosis for cystic fibrosis in Brittany, France. *V. Scotet[1], M. De Braekeleer[2], M. Roussey[3], G. Rault[3], P. Parent[3], M. Dagorn[3], H. Journel[3], A. Lemoigne[3], J.P. Codet[4], M. Catheline[4], V. David[5], A. Chaventre[2], C. Verlingue[1], I. Quere[1], B. Mercier[1], M.P. Audrezet[1], C. Ferec[1]*. 1) Department of Molecular Genetics, ETSBO - UBO, BREST; 2) Department of Genetic Anthropology and Demography - BORDEAUX; 3) Departments of Paediatrics of Brittany; 4) Departments of Nuclear Medicine of Brittany; 5) Department of Genetics, RENNES, FRANCE.

Cystic fibrosis (CF) is the commonest severe autosomal recessive disease in white population of Caucasian origin. Cloning of the gene responsible for CF and identification of its mutations with their ethnical and geographical distribution enabled to implement efficient strategies of molecular diagnosis and prevention. This study assessed the ten years of neonatal screening and prenatal diagnosis practice and studied their influence on CF incidence in Brittany, a French area of 2 millions of inhabitants of Celtic origin. Over the decade, 343.756 newborns were screened by measurement of immunoreactive typsin. 118 CF children were identified, leading to an incidence of 1/2913 which did not decrease significantly over the period. All the prenatal diagnoses performed over these ten years were registered : 62 diagnoses were realised in families related to a screened child, 102 in the relatives of a child born before the introduction of screening and 118 in couples who presented abnormal ultrasound scan. Through early detection of patients, neonatal screening allowed 12 couples to use prenatal diagnosis for future pregnancies whereas the screened child was still asymptomatic. Prenatal diagnosis, which identified 60 affected fetuses, allowed the birth of 222 healthy children and the termination of 54 pregnancies (a pregnancy termination level of 90%). Taking into account pregnancy terminations, incidence was 1/1999. However 50% of couples would not have extended their family without the existence of prenatal diagnosis; so 32 births were really avoided. Prevention strategies led to a modification of 21% in CF incidence in Brittany, and awaiting more efficient pharmacological or gene therapy-based treatments, prevention remains an important aspect in management of CF.

Program Nr: 2310 from the 1999 ASHG Annual Meeting

Mutation detection for Congenital Adrenal Hyperplasia: Analysis of 117 Dutch patients and comparison of existing methods for mutation screening in the CYP21 gene. *E.A. Sistermans, I.J. de Wijs, L.H. Hoefsloot.* Dept Human Genetics, Univ Hosp Nijmegen, Nijmegen, Netherlands.

Congenital Adrenal Hyperplasia (CAH) is a relatively common recessive disorder with a carrier frequency of approximately 1:50. It is characterised by excessive virilisation, and 70% of the classical cases show potentially fatal salt wasting. The disease is caused by mutations in the 21-hydroxylase (CYP21) gene located in the class III HLA region on chromosome 6. The gene is part of a 30 kb tandemly duplicated fragment, with an almost identical pseudogene (CYP21P) on the second segment. This particular structure determines the spectrum of mutations found in CAH, which consists of gross deletions/conversions and of small mutations derived from the pseudogene. We analysed 117 independent patients from the Netherlands for mutations in the CYP21 gene. In 103 patients, mutations were found on both alleles, whereas 7 patients had a common mutation on only one allele. No mutation was found in the remaining 7 patients, in all these cases the clinical diagnosis was uncertain. An overview of all mutations found will be presented. Analysis of deletions and large conversions in the CYP21 gene is generally performed by Southern blot analysis, whereas PCR-based techniques like allele specific PCR are used to detect the smaller mutations. At least ten independent tests have to be performed to cover all common mutations. Therefore, completion of mutation screening takes much time, which is not always available, for instance in the case of a pregnancy or when imbedded in a neonatal screening program. To overcome this problem, we started working on a new method that allows fast and reliable parallel detection of small mutations.

Program Nr: 2311 from the 1999 ASHG Annual Meeting

Genetic tests, screening and priorities in health care. *J.H. Solbakk, H.S. Hasan.* Centre for Medical Ethics, University of Oslo, Oslo, Norway.

Genetic testing has been developed in order to benefit individuals who are susceptible to genetic conditions which when manifested will either lead to the loss of quality of life or the loss of life or both. Genetic screening and the problem of priorities in relation to genetic testing will be the main focus of attention in this paper. A Socratic approach will be tried out, implying that several impertinent questions will be raised and some answers provided. The first question addressed is the purpose of genetic testing. The second question is which of these genetic tests should be given priority.

Five clusters of preliminary questions are also addressed: the question of the identity of different interest groups in the field of genetic testing (Who are they?); second, the nature of genetic counselling; third, the relation between legitimate and illegitimate interests in the field of genetic testing; fourth, the relation between self-evident and hidden concepts and values in the practice of genetic testing (What is meant by 'genetic disease', 'genetic susceptibility', to be 'at risk', 'low risk', 'high risk' etc.? Who has the authority to fill out the definitions of these concepts, and whose interests are at stake in the process of prescribing this new language? Last but not least, what are the potential effects and side effects of genetic testing and screening programs?

Norway was the first country worldwide to provide a system of priorities in healthcare in 1987. In various forms, other states and countries (Oregon, 1991; The Netherlands 1991; Sweden, 1991-95; Finland, 1994; Denmark, 1996 and New Zealand, 1992) have followed similar models, at least in terms of defining a core set of principles (severity of the disease, effectiveness, cost-effectiveness, and need) by which prioritisation decisions should be made.

We have applied these core principles to propose a model for genetic testing and screening programs.

Program Nr: 2312 from the 1999 ASHG Annual Meeting

A polymorphism in HFE promotes misdiagnosis of hereditary hemochromatosis. *M.J. Somerville, K.A. Sprysak, M. Hicks, B.G. Elyas, L. Vicen-Wyhony.* Dept Medical Genetics, Univ Alberta, Edmonton, AB, Canada.

Hereditary hemochromatosis (HH) is an autosomal recessive disorder of iron metabolism that is estimated to affect approximately 1 in 300 individuals. Two mutations in the HFE gene have been identified (C282Y and H63D) that contribute to HH (Feder et al. 1996), and screening for the C282Y mutation in particular, is used to identify carriers and affected individuals. We have obtained anomalous results in 8 out of 202 unrelated individuals tested for the C282Y mutation. PCR amplification followed by restriction digest in these individuals gave a C282Y homozygote digestion pattern with trace amounts of undigested DNA. Increased restriction enzyme and incubation time, as well as resampling of these cases did not resolve the anomalous results. Increased PCR stringency eliminated the normal product. Reanalysis of these cases using two alternative primers that flank the Feder et al. primer sites, gave a C282Y heterozygote pattern in all 8 samples. Sequencing of HFE revealed a G->A substitution (892+48G->A) in intron 4, 5 bases from the 3 terminus of the reverse primer site identified by Feder et al. This is not predicted to disrupt normal HFE splicing. In all 8 cases this mutation was on a non-C282Y, non-H63D chromosome. PCR using this reverse primer can result in dramatically reduced amplification of the polymorphic allele, such that a C282Y carrier will appear to be a C282Y homozygote. These 8 cases were obtained from a total of 43 unrelated C282Y (non-H63D) carriers, and in addition to 44 unrelated C282Y homozygotes. The estimated frequency of this allele in our population is, therefore, 8/43 = 0.186. If the assumption of homozygosity along with access to parental genotypes had been made in all polymorphic and homozygous cases, this would have led to an apparent 8% rate of non-paternity. The frequency of this polymorphism is high enough to warrant concern that the interpretation of homozygosity in these cases will result in an overestimate of the C282Y allele frequency, in misdiagnosis of this condition, in an underestimate of C282Y penetrance, and in an incorrect assumption of non-paternity in some families. Feder JN, et al. (1996) Nature Genet 13: 399.

Program Nr: 2313 from the 1999 ASHG Annual Meeting

An Empiric Method to Determine Sensitivity and Allele Frequency for Cystic Fibrosis Genetic Testing. *P. Srinivasan[1], J. McClellan[2], M.E. Nunes[2].* 1) University of Texas Medical Branch, Galveston, TX; 2) USAF Medical Genetics Center, Keesler AFB,MS.

The American College of Medical Genetics (ACMG) 1997 Statement on Genetic Testing for Cystic Fibrosis (CF) suggests that adequate experience with sensitivity and specificity in target populations be obtained prior to universal offering of preconception/prenatal CF genetic testing. The majority of *GeneTests*$_{TM}$ (*Helix*) listed laboratories which offer CF testing determine their sensitivity by adding individual allele frequencies derived from CF Consortium tabulated data for each mutation in their test panel. Reported sensitivities for Caucasians using equivalent mutation panels range from 80% to 90%. We report a simple empiric method to independently determine sensitivity and allele frequencies for CF mutations in clinical laboratories, regardless of the molecular methodology used. The predicted number of independently ascertained CF chromosomes in the test pool, *C*, can be determined from clinical information provided in the test requisition form and applying the following formula: $\mathbf{C = 2a + S(R \times b)_i + S(2pq \times c)_i}$ where *a* is the number of known CF cases, *R* the probability of being a carrier based on pedigree analysis (in most cases the coeffecient of relationship), *b* the number of at risk carriers at each probability, *2pq* the carrier frequency for a given ethnic population, and *c* the number of individuals without family history of CF of given ethnicity. Our molecular diagnostic laboratory offers a 21 CF mutation panel. A two year period covered 393 cases, which correcting for shared parental chromosomes represented 526 independent chromosomes. Applying the above formula predicts 84.6 independent CF chromosomes. From this sample, 67 mutations were detected (sensitivity = 67/84.6 = 79.20%): 61 DF508 (allele frequency = 61/84.6 = 0.728), two G542X (0.024), and one each R553X, N1303K, G551D,and R560T (0.012 each). Using the formula it should be possible for any laboratory to empirically determine overall test sensitivity. With a large enough sample size, specific sensitivities and allele frequencies for different ethnic and racial groups can readily be determined as well.

Program Nr: 2314 from the 1999 ASHG Annual Meeting

Predictive testing for the dominantly inherited corneal dystrophies. *H.S. Stewart[1], G. Black[1,2], G. Hall[1], D. Donnai[1], A.E.A. Ridgway[2], D. Craufurd[1]*. 1) Department of Medical Genetics, St Mary's Hospital, Manchester, UK; 2) Manchester Royal Eye Hospital, Oxford Road, Manchester, UK.

Introduction: The autosomal dominantly inherited corneal dystrophies (CD's) form a heterogeneous group, clinically and genetically. They vary in their age of onset and the severity of the problems they cause. Reis-Bucklers dystrophy has a childhood onset of recurrent, painful corneal erosion and there is a rapid loss of visual acuity necessitating corneal transplantation by the second decade. In contrast, late-onset lattice CD causes less severe visual problems, though corneal transplantation may eventually be necessary due to loss of visual acuity. Since the BIG-H3 gene on chromosome 5q31 has been shown to cause a number of CD's, genetic testing prenatally, pre-symptomatically and to confirm a suspected diagnosis are all theoretically possible. Purpose: We aimed to assess the psychological burden of being at risk in people at 50% risk of CD by virtue of their family history and assess their attitudes to predictive testing. We compared this with other late onset diseases such as Huntington's disease and familial cancer, for which predictive testing is possible. Patients with CD are primarily managed by ophthalmologists. Comparisons were made with the attitudes of the people at risk and the corneal surgeons. Methods: Individuals from 24 CD families were invited to complete telephone questionnaires. Postal questionnaires were sent to 119 surgeons. Results: Being at risk of developing CD is not a major psychological burden. Predictive testing was favoured 100% responding patients and 80% of surgeons. Advantages of testing were cited as career planning and to clarify the risk to children. Ethical considerations such testing in childhood and testing people at 25% risk were poorly understood. Conclusions: The International Guidelines for predictive testing, based on the models of Huntington's disease and familial cancer, may need to be modified for late onset diseases that cause less psychological burden. Interdisciplinary debate is necessary to devise such modified guidelines.

Program Nr: 2315 from the 1999 ASHG Annual Meeting

Development of an improved molecular assay for detection of mutations causing Tay-Sachs disease. *T.L. Stockley, G. Koultchitski, P.N. Ray.* Department of Pediatric Laboratory Medicine, The Hospital for Sick Children, Toronto, ON, Canada.

Tay-Sachs disease is an inherited lysosomal storage disorder caused by deficiency of the enzyme hexosaminidase A and is most prevalent in the Ashkenazi Jewish population (carrier rate 1/30). Carrier screening for Tay-Sachs is available by biochemical analysis of hexosaminidase activity in blood. However, the biochemical assay is complicated by several difficulties, such as inaccurate results for women who are pregnant or using birth control pills. As well, individuals who carry non-pathogenic pseudodeficiency mutations are reported as Tay-Sachs carriers by use of the biochemical assay. Due to these difficulties, molecular analysis of mutations in the HEXA gene is an important compliment to biochemical screening. However, many common current methods for detection of mutations in HEXA are laborious and are not suitable for molecular analysis of large numbers of individuals.

We have developed a molecular assay which simultaneously detects five common HEXA mutations. The mutations detected in this assay include three pathogenic mutations, 1278insTATC, IVS12(+1)G®C and 805G®A (G269S), which account for 96% of HEXA mutations in the Ashkenazi Jewish population. The assay also detects two pseudodeficiency mutations, 739C®T (R247W) and 745C®T (R249W), which are common in the non-Jewish population. The assay is based on the Amplification Refractory Mutation System (ARMS), which relies on the fact that primers which have a mismatch at the 3' terminal nucleotide will not function in PCR. We have adapted the ARMS methodology to detection of HEXA mutations by designing fluorescent dye labelled ARMS primers specific to either the normal or the mutant DNA sequence for each of the five mutations to be detected. This assay is an improvement on previous molecular assays for Tay-Sachs as it simultaneously detects five HEXA mutations in a single tube, thus reducing the labor and cost associated with molecular testing for Tay-Sachs disease.

Program Nr: 2316 from the 1999 ASHG Annual Meeting

Detection of genomic mutations and polymorphisms of Protein C (PROC) gene using denaturing high performance liquid chromatography (DHPLC). *M.R. Taliani[1,2], S.C. Roberts[1], R.K. Pruthi[1], W.L. Nichols[1], J.A. Heit[1]*. 1) Hematology Research, Mayo Clinic and Foundation, Rochester, MN; 2) Institute of Internal and Vascular Medicine, University of Perugia, Italy.

Background: Genomic abnormalities responsible for the deficiency of PROC have been extensively studied in the last years providing important insights into the PROC structure-function relationship. Unlike other hereditary defects of antithrombotic mechanisms, the PROC gene abnormalities are heterogeneous. Thus it is difficult and expensive to identify the genomic abnormality in a given patient, since each exon has to be amplified and sequenced. At present, there are a number of available techniques wich allow screening large populations for sequence variations, but they present some disadvantages such as lack of sensitivity, high costs and labor intensity. DHPLC has been recently described as a new, rapid, automated, scanning method of unknown mutations. **Objective**: To evaluate the sensitivity and the specificity of DHPLC as a method for rapid screening of unknown mutations within the PROC gene of patients with PROC deficiency. **Study population**: Patients (n=24) with a history of venous thromboembolism referred to the Mayo Clinic Special Coagulation Laboratory and found to have PROC deficiency. **Methods**: Protein C activity was determined by chromogenic assay using Protac and protein C antigen level was determined by ELISA. For each patient, a minimum of 4 kb of the PROC gene was amplified by PCR and sequenced using fluorescence-based Perkin-Elmer ABI methodology. After a denaturing and reannealing step, 5-10 ml of each PCR product were analyzed by DHPLC (Transgenomic Wave DNA Fragment Analysis System). A blinded analysis of the chromatograms obtained from the DHPLC was performed by an independent reader. These results were then compared to the sequence data. **Results**: On the basis of number, width and separation of chromatogram peaks, a total of 47/47 heterozygous mutations and heterozygous polymorphism patterns were correctly identified as were 193/193 homozygous wildtype alleles. **Conclusion**: We conclude that DHPLC is a highly sensitive and specific method for the rapid detection of unknown mutations.

Program Nr: 2317 from the 1999 ASHG Annual Meeting

The Effects of Race, Maternal Age, Income, and Timing of First Prenatal Care on the Utilization of Prenatal Diagnosis and the Percent Reduction of Live Births with Down Syndrome in California, 1992 - 1995. *J.M. Tepe[1], C.A. Huether[1], C. Torfs[2], L. Feuchtbaum[3]*. 1) Biological Sciences, University of Cincinnati, Cincinnati, OH; 2) California Birth Defects Monitoring Program, Emeryville, CA; 3) Genetic Diseases Branch, California Department of Health Services, Berkeley, CA.

Two significant factors that affect the incidence rate of Down syndrome are maternal age and utilization of prenatal diagnosis with elective termination. Additionally, there are sometimes differences in incidence rates seen among races. These differences most likely occur due to the above two factors, with the differing utilization rates most likely due to varying sociologic factors.

This study examines the effects of race, maternal age, income, and timing of first prenatal care on the utilization rates of prenatal diagnosis and their resulting effects on the percent reduction of Down syndrome. As a retrospective study, the data have been abstracted from two Californian databases, the California Birth Defects Monitoring Program and the Genetic Diseases Branch of the Department of Health Services for the time period 1992 - 1995. By combining these two sources, a comprehensive cohort of all fetuses with Down syndrome is obtained. The four-year period yields 1,441 live births and 842 terminations. Preliminary analysis indicates that the overall reduction in live births with Down syndrome was approximately 30.2% over the four years of the study. The percent reductions by race categories are as follows: Asian American, 46.9%; African American, 22.7%; Hispanic, 15.1%; Caucasian, 51.9%; and Other, 34.6%. The magnitude of effect of each of the four independent variables, and their interactions, on both the utilization rates of prenatal diagnosis and the percent reduction of Down syndrome will be presented. The results will provide an increased understanding about the differences in utilization of prenatal diagnosis among races. Given the impact of individuals with Down syndrome on the health care and educational establishments, these results are of importance to health and education providers.

Program Nr: 2318 from the 1999 ASHG Annual Meeting

The Effect Of One Genetic Support Group On Research For A Rare Disease. *S.F. Terry[1], P.F. Terry[1], L.G. Bercovitch[1,2].* 1) PXE International, Inc, Sharon, MA; 2) Department of Dermatology, Brown University, Providence, RI.

A patient support organization can accelerate and focus research on a rare disorder. Research on a rare genetic disorder is limited by lack of data about its manifestations, natural history and epidemiology. Often the very lack of large numbers 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 support organization founded in 1995, initiates, supports and funds research. Also providing support for affected members and their families, it has focused and accelerated research on this disorder. As a lay support organization it is has established a database of 1500 affected individuals and 250 pedigrees, created a blood and tissue bank housing 900 DNA samples and 90 tissue samples, collected detailed epidemiological data from 400 individuals, initiated several research projects, and described these and other projects to patients in a direct and expedient manner. PXE International has expended 80% of its operating budget on direct and indirect costs of research. It sponsored regular research meetings for studying PXE and related basic science to encourage expedient definition of problems and to open new avenues for further research.

The results of the efforts made by PXE International are a 15 laboratory consortium, an International Centennial Meeting on Pseudoxanthoma Elasticum cosponsored by the NIH, 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 support groups using this model can greatly benefit both research and patient populations, furthering both the common and divergent goals of each.

Program Nr: 2319 from the 1999 ASHG Annual Meeting

Implication of Screening for FMR1 and FMR2 Gene Mutation in Individuals with Nonspecific Mental Retardation in Taiwan. *C.C. Tzeng[1,2], P.Y. Tzeng[2], H.S. Sun[2], R.M. Chen[1,2], S.J. Lin[2,3].* 1) Departments of Pathology; 2) Molecular Medicine; 3) Pediatrics, National Cheng-Kung University Hospital, Tainan, Taiwan, R.O.C.

Fragile X syndrome (FXS) is the most common familial mental retardation (MR), attributable to (CGG)n expansion in the FMR1 gene. FRAXE is less frequent, associated with a similar mutation of FMR2 gene. Both disorders are as yet not treatable but can be prevented by prenatal genetic examination. However, screening of all pregnant women for carriers is thus far technically not cost-effective. In this study we attempted to ascertain the prevalence of both disorders in MR population in Taiwan, as well as to develop a method to effectively find carriers. We screened 321 nonspecific MR patients for the FMR1 and FMR2 mutation. Four (1.9%) out of 206 males and one (0.9%) in 115 females were identified as FXS patients. All four FXS males could be detected by Southern blot analysis, as well as by a simple nonradioactive PCR. None of the 206 males had FMR2 full mutation. This confirmed the low incidence of the FRAXE in Chinese. FXS patients appears to be more prevalent among mildly MR, since 4 of the 5 FXS were from the 115 mildly MR (3.5%) and only one from the other 206 severely MR (0.5%). All five FXS were maternally inherited. Further search of other family members for carriers was rather resistant. Worthy to note is that none of these mothers had discernible premarital family history of MR. Thus the negative family history could not preclude a woman from being a carrier. How to identify female carriers of childbearing age, beyond the scope of family history, is thus worthy to be further explored. We here demonstrated a simple and inexpensive PCR procedure suitable for wide screening of males, at a cost of US$ 6.00 per case. It could reliably classify males as possible FXS, PM, or normal. Although normal transmitting males have no immediate risk of having a diseased child, tracing them and FXS patients would easily identify more carrier women at or before childbearing age for more preventive measures.

Program Nr: 2320 from the 1999 ASHG Annual Meeting

Denaturing High Performance Liquid Chromatography (DHPLC) for PMM2 mutation screening in CDG type 1A patients. *A. Uller[1], H. Stibler[2], B. Kristiansson[3], J. Wahlstrom[1], T. Martinsson[1]*. 1) Dept Clin Genet, Gothenburg Univ, Sahlgrenska Univ Hosp, Gothenburg, Sweden; 2) Dept Neurology, Karolinska hosp, Stockholm, Sweden; 3) Dept Pediatr, Sahlgrenska Univ Hosp, Gothenburg, Sweden.

The phosphomannomutase 2 gene (PMM2) has been identified as the carbohydrate-deficient glycoprotein syndrome type 1A gene (CDG type 1A). The gene spans 8 exons and 741 bps of coding DNA. Previously, we have identified 20 different mutations in the PMM2 gene using mutation screening with SSCP (single-stranded conformation polymorphism) and sequencing of DNA from 61 CDG type 1A patients. Since seven of these could not be detected by SSCP in this first study, we were not satisfied with the sensitivity of the mutation detection technique used. Thus, we wanted to investigate if DHPLC was a more suitable mutation screening method for PMM2. DHPLC was set up for PMM2 by optimizing eight different PCR fragments, one fragment for each exon. The mutation detection was optimized empirically with PCR fragments from wild-type controls. First, wild-type samples were run at a universal-gradient and after modifying and shortening of the gradient, also run at ten different temperatures, 50-70oC with 2 degrees interval, to enable setting of the temperature with the highest resolution. Then, PCR products with known mutations from the previous study, were loaded and the results were compared to the wild-type chromatograms for aberrations. We detected 19/20 mutations with DHPLC. Several mutations not detected by earlier techniques were readily detected by dHPLC. In conclusion, the use of DHPLC here shown to detect 95% of the mutations in the PMM2 gene is likely to provide a powerful tool for diagnostics for CDG type 1A patients and families in terms of sensitivity, reliability and automation.

Program Nr: 2321 from the 1999 ASHG Annual Meeting

Genetic Diagnosis Challenge: Experience at the National Institute of Pediatrics in Mexico city. *N. Urraca, MC. Esmer, V. Del Castillo.* Genetics, Institute National Pediatrics, Mexico, city, Mexico.

The diagnosis of a genetic disease depends on the capacity of the geneticist and on the availability of biochemical, radiological, chromosomal and molecular tests. We studied 400 charts to investigate our frequency of undiagnosis, we analyzed 1992 and 1996 to asses whether there was a change on frequency with the access to computerized systems and to molecular tools. The mean age of onset was 8.8 months, and the average on patients attendance to consultation was at 30.2 months of age. In 68% of all patients had a diagnosis, 37% of them were diagnosed by other specialists and the remainder at the genetic consultation: history and physical examination helped on 26%, laboratory studies on 34%, the academic meetings, literature search and the computerized systems on minor grade. The diagnostic frequency was significatively high in 1992, more patients arrived diagnosed in 1996, probably on the later years the easier and recognizable disorders are not seen, therefore it is possible that the complexity of the disease is increasing at this level of attention. Almost one third of the patients with a diagnosis had a mendelian disorder or an entity with unknown etiology. 108 different diagnosis were made on 274 patients. The most prevalent were oculo-auriculo-vertebral spectrum (OAVS), non-syndromic cleft lip and palate (CLP), Down's syndrome, myelomeningocele and neurofibromatosis. Although 30&% of the patients with unknown diagnosis had a suspicion disorder, it couldn't be confirmed probably because the patients didn't return to complete their studies or due to the absence of confirmatory resources. 11.9% had only mental retardation, which itself represents a clinical challenge to establish a etiologic diagnosis needing the performance of magnetic resonance and molecular tests that are not always available. 23% have abnormalities that probably encompass a syndrome not yet described. This study confirms the need for the clinical geneticist to have complementary tools that improve the study of these patients in order to lesser the anxiety of the family.

Program Nr: 2322 from the 1999 ASHG Annual Meeting

A DGGE system for comprehensive mutation screening of the complete coding regions of BRCA1 and BRCA2 outside exons 11. *A.H. van der Hout, I.M. Mulder, Y. Wu, P. van der Vlies, M. Huisman, J.C. Oosterwijk, C.H.C.M. Buys, R.M.W. Hofstra.* Medical Genetics, University of Groningen, Groningen, The Netherlands.

A family history of breast and/or ovarian cancer is one of the strongest risk factors for breast cancer. A substantial portion of all hereditary breast and/or ovarian cancer is associated with germline mutations in BRCA1 and BRCA2. We have used PTT for screening on a routine basis patients from breast/ovarian cancer prone families for truncating mutations in the large exons (exon 11 of BRCA1 and exons 10 and 11 of BRCA2). Moreover, we screened by PCR analysis for the in the Dutch population frequently occurring deletions of exon 13 or 22 of BRCA1. Mutations were found in 37/218 families (17%). To scan the remaining part of the coding sequences of both genes for all possible small mutations we have designed a DGGE system in which we divide the coding region of both genes outside exon 11 plus the first and last 500 bp of the exons 11 into 81 amplicons. These are combined in 22 pools which are subjected to DGGE in a 9% PAA gel for 16 hours, 120V, with a urea/formamide gradient of 20-65% at 59°C (BRCA1) or 30-65% at 55°C (BRCA2). We use DGGE because we have shown in other diseases that with an appropriate design the sensitivity of this technique is virtually 100%. With this system all known Dutch mutations in BRCA1 and BRCA2 outside exons 11 (n=16) were indeed easily detected. Until now we screened 14 families in which no mutation was found with PTT/deletion analysis. In three families frameshift mutations were found, one of these not reported previously in the BIC database. Moreover, a number of unclassified variants and polymorphisms were detected. Thus, a combination of PTT and this DGGE system allows a relatively easy and fast scanning of the complete coding sequences of BRCA1 and BRCA2 for mutations on a routine basis.

Program Nr: 2323 from the 1999 ASHG Annual Meeting

PCR-based methylation testing of archived fixed cell suspensions from individuals previously tested by cytogenetics/FISH for Prader-Willi or Angelman syndromes. *M. Velinov[1], H. Gu[1], M. Genovese[1], C. Duncan[1], W.T. Brown[2], E. Jenkins[1]*. 1) Dept Cytogenetics; 2) Dept Human Genetics, IBRDD,Staten Island.

Until recently most diagnostic evaluations for Prader-Willi (PWS)and Angelman (AS)syndromes consisted of a combination of high resolution banding and FISH analyses to look for microdeletions in the proximal long arm of chromosome 15. However cytogenetic and FISH microdeletion analysis can only identify about 70% of individuals suspected for PWS or AS. In contrast, DNA methylation testing identifies nearly 100% of PWS and 75% of AS individuals. Thus methylation testing was recommended for individuals with suspected PWS or AS and negative FISH analysis (AJHG, 58: 1085-1088, 1996). We have carried out methylation testing on standard cytogenetic fixed cell suspensions from people previously tested for PWS or AS with negative cytogenetic/FISH findings. These specimens were refrigerated after FISH analysis for 0-4 years. We used two PCR-based methylation tests: methylation specific PCR (mPCR), which was reported previously (Kubota et al. Nat. Genet.;16:16,1997) and a bisulfite restriction analysis(BRA) method that we recently developed (Velinov et al. Gen.Med.1(2):75,1999,A124). The mPCR and BRA tests were initially standardized using specimens from individuals with known PWS and AS as well as normal controls. The mPCR method was successful in testing 15 out of the 16 fixed cell suspensions. One specimen failed to amplify with the primers used, possibly due to insufficient amount/quality of the genomic DNA tested. All fixed cell suspensions showed normal methylation patterns, thus ruling out PWS or AS. Since BRA was only successful with larger amounts of template, we were able to test only 5 out of the 16 specimens studied. All the BRA results supported the mPCR findings. We thus showed for the first time that archived fixed cell suspensions from individuals suspected for PWS/AS that were negative for cytogenetic/FISH microdeletions,can now be re-evaluated with PCR-based methylation tests without requesting additional specimens from the previously studied individuals. This work was supported in part by the NYS Office of Mental Retardation and Developmental Disabilities.

Program Nr: 2324 from the 1999 ASHG Annual Meeting

Multiplex PCR for Down's diagnosis. *D.Y. Venable[1], X. Li[1], X. Hou[2], L. Zhang[2], G. Feng[2], F. Song[2], B. Qi[2], J. Han[1].* 1) Genaco Biomedical Products, Inc., Huntsville, AL; 2) ShenYang Maternal Child Care Hospital, Liaoning, PR.China.

Down's syndrome is the most common chromosome disease. In the West, prenatal diagnosis is relying on cytogenetic analysis. This method, however, is not widely available in many developing countries. In China, for example, over 90% of the population still do not have access to prenatal genetic services. The limitations can be technical or economical. Technically, lack of reliable products and proper training lead to a low amniotic cell culture success rate (less than 75%); Economically, if imported products (such as culture media and consumables) were used, most consumers could not afford the service. To make prenatal Down's diagnosis more accessible to people in developing countries, we have developed an accurate, easy, and affordable molecular diagnostic kit. The kit used multiplex PCR to amplify three chromosome 21 specific polymorphic loci. The biotin-labeled PCR products were separated with gel electrophoresis and transferred onto nylon membrane for color detection. Relatively high detection rate is achieved by this multi-allelic system. Down's diagnosis can be made either qualitatively (about 95% of Down's patients can be recognized as having three alleles) or quantitatively (two alleles, but with a 2:1 intensity ratio). The kit is easy to use and its application requires only a thermal-cycler and gel apparatus.

Program Nr: 2325 from the 1999 ASHG Annual Meeting

Health-related quality of life indices for breast cancer treatments and genetic testing options. *S. Verma[1], M. Cappelli[2], L. Surh[2], L. Humphreys[2], D. Logan[1], A. Hunter[2], J. Allanson[2].* 1) Ottawa Regional Cancer Centre - Genreal Division; 2) Children's Hospital of Eastern Ontario.

In establishing decision models for the treatment and prevention of breast cancer, it is important to evaluate the health-related quality of life afforded to patients by such interventions. The objectives of the present study were: (i) to characterize women's preferences for breast cancer treatments and BRCA1/BRCA2 testing, using the rating scale and standard gamble techniques for eliciting health state utilities ranging between 0 (death) and 1 (perfect health); and (ii) to identify factors associated with these quality-of-life indices. Data were collected from: 60 women diagnosed with breast cancer before 50 years of age; 58 unaffected, high-risk female relatives of women with early-onset breast cancer; and 51 unaffected women in the general population. Multivariate analyses of variance (MANOVAs) and factorial analyses of variance (ANOVAs) revealed that health state utilities did not differ according to group membership or whether or not participants intended to undergo genetic testing in the near future. There was, however, a trend for married women to evaluate certain health states more highly than unmarried women, and for women with a higher level of education to value preventive drug therapy more highly than women with less than a university education. It was concluded that women's preferences for various breast cancer treatment and prevention options are not related to the degree of experience they have with the disease, nor are they related to women's choices about receiving BRCA testing in the near future. Certain demographic factors may play a role, however. Results of this study provide valuable information regarding the quality of life attributed by affected and unaffected women with respect to breast cancer genetic testing and treatment outcomes.

Program Nr: 2326 from the 1999 ASHG Annual Meeting

Identification of Novel Mutations in Hispanic Cystic Fibrosis Patients. *J. Wang[1], M. Bowman[2], E. Hsu[2], K. Wertz[2], L. Wong[1]*. 1) Inst Molecular & Human Gen, Georgetown Univ Medical Ctr, Washington, DC; 2) Department of Pediatrics, Children's Hospictal Los Angeles, California.

Cystic fibrosis (CF) is a common autosomal recessive genetic disorder, with a carrier frequency of about 1:25 in Caucasians. More than 800 mutations have been found in the CF gene. The mutation spectrum varies among different ethnic groups. In Ashkenazic Jewish, five mutations account for more than 90% of CF alleles. However, only about 58% of Hispanic CF mutations have been identified. Here we report the use of temporal temperature gradient gel electrophoresis (TTGE) method to scan the mutations in CFTR gene. 32 Hispanic CF patients who have been screened for a panel of 70 common mutations and have at least one unidentified mutant allele were studied by TTGE. Each of the 27 exons of CFTR and the flanking intron regions was PCR amplified followed by TTGE analysis, which separates the homo- and heteroduplexes formed between mutant and wild type DNA based on their melting behavior. Seven novel mutations were identified, in addition to two polymorphisms. Three of these mutations were found heterozygous in more than two unrelated CF Hispanic patients. They are 3876delA, 935delA and 406-1G>A. 3876delA was found in seven patients and each of the other two in two patients, respectively. Both 3876delA and 935delA mutations are associated with growth retardation, lung and intestine impairment and pancreatic insufficiency. In addition, both patients heterozygous for 935delA had meconium ileus. Other symptoms include early deaths, liver disease, cor pulmonale and diabetes. These clinical findings suggest that 3876delA and 935delA mutations are associated with severe CF phenotype. Our results demonstrate that TTGE is an effective method for screening unknown sequence variations. The 3876delA mutation was found in about 15% of our Hispanic patients. Both 935delA and 406-1G>A are recurrent. This information will be useful for genetic counseling to Hispanic patients. *cor pulmonale*.

Program Nr: 2327 from the 1999 ASHG Annual Meeting

Public attitudes regarding the donation and storage of blood specimens for genetic research. *S.S. Wang[1], F. Fridinger[1], K.M. Sheedy[1], H. Linnan[2], M.J. Khoury[1]*. 1) Office of Genetics & Disease Prevention, CDC, Atlanta, GA; 2) Office of Communication, CDC, Atlanta, GA.

As sequencing of the human genome progresses, the demand will increase for population-based genetic research to assess allele frequencies and associations between specific polymorphisms and human diseases. Such research will include large population-based cross-sectional and cohort studies that collect and store blood samples for DNA studies. In order to assess the current climate towards donating and storing blood for genetic research, the authors examined data from the 1998 American Healthstyles Survey. Individuals participating in this survey of health attitudes and behavior are representative of adults in the U.S. with regard to age, gender, marital status, race, income, region, household income, and population density. As part of this extensive survey, a series of questions regarding blood donation and storage for genetic research was posed to the participants. Of 3,130 survey participants, 2,621 (84%) completed questions regarding blood donation and use of stored blood for genetic research. We found that 42% were in favor of both blood donation and long term storage for genetic research; 10% were willing to donate blood for genetic research but not for long term storage; 27% were not willing to donate blood but would consider it under certain circumstances (confidentially or anonymously); and 21% were not willing to donate or store blood for genetic research under any circumstance. Factors affecting the likelihood of respondents to be in favor of blood donation for genetic research and long term storage include: higher education, higher income, positive family history for a genetic disorder, individuals living in the pacific and mountain regions of the U.S., Caucasians, and individuals 30-64 years old ($p<0.05$). These results indicate a wide range of public opinion on donation and storage of blood specimens for genetic research. Further understanding of the multiple factors which contribute to attitudes regarding genetic research will contribute to future actions in communicating genetic research goals to the public and recruitment for population-based genetic studies.

Program Nr: 2328 from the 1999 ASHG Annual Meeting

SCA 8 in the Scottish population. *J.P. Warner, L.H. Barron, M.E. Porteous.* Molecular Genetics Service, Western General Hosp, Edinburgh, Scotland.

A CTG expansion in the 3' UTR of a gene in 13q21 has recently been described as the cause of SCA 8. This (CTG)n expanded sequence is preceded by a polymorphic stretch of (CTA)n. We describe PCR amplification methods which allow accurate independent size determinations of both trinucleotide repeats. Allele frequencies for both repeats are presented for the Scottish normal population. New TP-PCR primers are presented which help identify the presence of large difficult to amplify expanded alleles. Since 1989 a cohort of over 100 individuals with cerebellar ataxia of unknown origin has been referred to us by local neurologists for testing. The majority of these patients have no family history of ataxia and have previously been tested for SCA 1, 2, 3, 6, 7, DRPLA and Friedreich's ataxia. We present clinical details for individuals found with a combined CTG/CTA repeat at the upper end of the reported affected range for SCA 8. SCA 8 is currently the most common cause of spino cerebellar ataxia in our population.

Program Nr: 2329 from the 1999 ASHG Annual Meeting

Mutations and associated polymorphisms in Saudi -thalassaemia patients. *A.S. Warsy[1], M.A. El-Hazmi[2].* 1) Biochemistry, King Saud University, Riyad, Saudi Arabia; 2) Medical Biochemistry, King Khalid Hospital,King Saud University,Riyadh,Saudi Arabia.

The -thalassaemias are frequently encountered in the Saudi population with a diverse clinical picture. We investigated the -thalassaemia mutations in 98 Saudi patients using ARMS, dot blot analysis and DNA sequencing, using ABI 310 prism Sequencer. The spectrum of the mutations covered IVS-I-110 (GA), IVS-II-I (GA), IVS-I-5 (GC), CD 39 (CT), IVS-I-3' (-25bp) and CD 6 (-A), CD 8 (+1), Cap+1 (-25 bp), CD 5 (CCTC--), IVS-I-6 (TC); IVS-I-1 (GA) and IVS-II-848. Of interest is the findings of extensive polymorphic sites. The polymorphic sites identified included: IVS-II-26, IVS-II-244, IVS-II-76, CD 2, IVS-II-81, IVS-II-666, IVS-II-16 and IVS-II-74, as heterozygotes, homozygotes and double homozygous states. In addition, multiple polymorphic sites, upto 6 polymorphic sites in the same patient, were frequently encountered with variable prevalence. The paper will highlight the molecular pathogenesis of -thal. in Saudis and the associated multiple polymorphisms. The modulation of clinical presentation of -thalassaemias in Saudis, a phenomenon of frequent occurrence in Saudi sickle cell disease, and the relation to the polymorphism will be discussed.

Program Nr: 2330 from the 1999 ASHG Annual Meeting

Comparison Between Two Methods For Human Buccal Cell DNA Extraction: A High Throughput Method Versus One That Generates Ultra-pure DNA. *J.B. Watson, H. Grunenwald.* Epicentre Technologies, Madison, WI., U.S.

The use of human buccal cells as a convenient, non-invasive source of DNA has increased dramatically over the last decade. In this study we compared two methods for isolating DNA from buccal cells. The first uses a heat and amplify method that generates PCR amplifiable DNA using an extraction solution containing a PCR enhancer and a PCR inhibitor chelator. The yield using this method varies from 1-10 g of DNA and generates enough sample for up to 250 PCR amplifications. The primary advantage of this system is that the entire extraction is performed within a closed tube, which minimizes hands on manipulation, assures no cross contamination during the extraction process and allows hundreds of samples to be processed in a single day. We compared this heat and amplify method with a ultrapure protocol that generates highly purified DNA from buccal cells using a simple salting out procedure that does not require hazardous reagents. DNA yields using this method were also between 1-10 g of DNA; but the DNA was free from contaminating protein as assayed by O.D. 260/280 ratios. The primary advantage of the ultrapure method compared to the heat and amplify method was that the DNA is precipitated and therefore can be diluted to any desired concentration after isolation. To determine which method is preferable for different applications, PCR amplifications were performed using primers that amplified regions of the Fragile X gene, the hemochromatosis gene, the cystic fibrosis transmembrane receptor gene, and the apolipoprotein E gene. To determine their usefulness in forensics applications a set of short tandem repeat primers were amplified. It was found that PCR amplicons for all the genes could be generated using either method. The ultrapure DNA protocol generated more consistent PCR bands suggesting that if the PCR amplification is from a difficult template such as those with high GC content, the protocol that generates clean DNA is preferred. If the PCR will be performed on a large number of samples, and the PCR has been well-optimized, then the simpler heat and amplify method would be more efficient for these high throughput or automated applications.

Program Nr: 2331 from the 1999 ASHG Annual Meeting

A reverse hybridisation assay for molecular genetic diagnosis of familial mediterranean fever (FMF). *A.S. Weinhaeusel[1], C. Oberkanins[2], F. Kury[2], O.A. Haas[1].* 1) CCRI, St. Anna Children's Hospital, Vienna, Austria; 2) ViennaLab Labordiagnostika GmbH, Vienna, Austria.

Familial Mediterranean Fever (FMF; MIM 249100) is an autosomal recessive inherited disorder that is characterized by short, recurrent bouts of fever accompanied by pain in the abdomen, chest, or joints, and an erysipela-like erythema. In addition, renal amyloidosis can develop in patients with FMF. With a heterozygote frequency of 1/5 to 1/30 in the mediterranean area, the disease is widespread in North African, Jewish, Armenian, Turkish and Arab populations and individuals with their ethnic origin in these countries. FMF is caused by point mutations in the marenostrin gene located on chromosome 16(p13). The currently known mutations in exons 2, 5 and 10 cover at least 94 percent of disease-relevant mutations. Owing to the rather unspecific clinical symptoms, molecular genetic analysis significantly improves the diagnosis of this disease and, by that, facilitates correct treatment of homozygous and compound herterozygous mutation carriers with colchicine. Based on these prerequisites, we have developed a rapid and reliable assay for the molecular genetic detection of the most common FMF-mutations. The test is based on the reverse-hybridization principle and covers more than 94% of mutations that are found in the Mediterranean populations. Some essential improvements over published procedures have been introduced to facilitate the use of this test as a tool for routine diagnosis: (I) a single multiplex amplification reaction is used to amplify exon 2, 5 and 10 of the FMF gene; (II) premade, ready-to-use test strips are provided that contain oligonucleotide probes for each wild type and mutated allele immobilized as parallel lines. It is therefore possible from one single strip to discriminate heterozygous, compound heterozygous, and homozygous individuals; (III) the entire procedure from blood sampling to the identification of mutations can easily be carried out within a single working day. The test may be automated on equipments, such as the TECAN profiBlot.

Program Nr: 2332 from the 1999 ASHG Annual Meeting

The use of cheek brush DNA and allele specific oligonucleotides in screening for the APC I1307K allelic variant in Ashkenazim. *A. Whetsell[1], M. Wright[1], D. Shrag[2], J. Garber[2], S. Syngal[2], G.A. Miller[1], R.J. Pomponio[1].* 1) Molecular Profiling Laboratory, Genzyme, Framingham, MA; 2) Dana Farber Cancer Institute, Boston, MA.

The I1307K allelic variant in the Adenomatous Polyposis Coli gene (APC) has been reported to occur in about 6-7% of Ashkenazi Jewish individuals has been implicated as promoting increased susceptibility to colorectal and breast cancer.

As part of an ongoing study, we utilized DNA extracted from standard cytology cheekbrushes collected from 448 unrelated Ashkenazi Jewish patients with a history of either colorectal cancer, polyposis or adenomas. The samples were PCR amplified, dot-blotted, and analyzed for the presence or absence of the I1307K allele using allele specific oligonucleotides (ASOs). Eleven patients failed to PCR amplify, possibly due to improper cheek cell collection. Thirty-eight individuals (8.7%) were found to carry the I1307K allele variant, and of these, 2 were homozygous.

This data demonstrates the feasibility of large scale testing for the I1307K allele using bucal swab deried DNA.

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Program Nr: 2333 from the 1999 ASHG Annual Meeting

Scorpions™ primers - a novel method for use in single-tube genotyping. *D. Whitcombe, S. Kelly, J. Mann, J. Theaker, C. Jones, S. Little.* AstraZeneca Diagnostics, Northwich, Cheshire, UK.

The application of SNP and point mutation analysis in a clinical setting needs simple, cheap and reliable test formats. With this in mind we have developed a novel genotyping method which requires only a single addition of a DNA sample to a single tube to perform the assay.

The method employs PCR primers with integral tail sequences which we call Scorpions primers. The tail is complementary to part of the primer extension product, carries a fluorophore quencher pair and incorporates a blocking sequence to prevent it being copied during PCR. This allows the Scorpions primer to signal when it has been incorporated into the appropriate amplicon via a uni-molecular rearrangement which separates the fluorophore from the quencher. The system has substantial benefits in terms of kinetics, thermodynamics, assay design and reliability. We have combined Scorpions signalling with ARMS™ allele specific amplification to generate single tube genotyping tests.

The method has been used to develop single tube genotyping tests for SNPs and point mutations in a number of genes including CFTR, AAT, IL1a, BRCA1,VCAM, MLH1, HFE and also several non-genic SNPs. The utility of the system has been demonstrated by performing over 3,000 genotyping reactions.

Program Nr: 2334 from the 1999 ASHG Annual Meeting

The APEX SNP Identity Chip and the *IDENTI*PLEX™ STR Identity Testing System. *L.D. White[1,2], J.J. Tollett[2], L.A. Liles[1], J.P. Teske[1], J.H. Warren[1], R.W. Staub[1]*. 1) Molecular Genetics, Identigene, Inc, Houston, TX; 2) Dept. Molecular & Human Genetics, Baylor College of Medicine, Houston, TX.

We have developed a 50 locus microarray for human identity testing at Identigene, Inc. based upon single nucleotide polymorphisms (SNPs). The 50 SNPs were chosen based on reported allele frequencies of approximately 50%. Using Arrayed Primer Extension (APEX) on the 50 locus chip, we are able to genotype individuals at 50 loci simultaneously. APEX involves the template specific extension of an oligonucleotide primer (bound to a silanized glass surface) using a thermostable DNA polymerase and fluorescently labeled ddNTP terminators. Amplification of the 50 SNP sites was performed with multiplex PCR technology (5 reactions/50 sites). The fluorescently labeled SNP sites were detected on an Avalanche Microscanner™ (Amersham Pharmacia Biotech/Molecular Dynamics) that was modified for 4-color detection. Current testing protocols at Identigene, Inc. include multiplex PCR amplification of 11 short tandem repeat (STR) loci with fluorescently labeled primers and electrophoresis of the resulting gene fragments on an ABI Prism™ 377 Automatic DNA Sequencer. We tested 20 families from the American Black population on both the 50 SNP Identity Chip and on the *IDENTI*PLEX™ STR ABI Prism™ 377 System. Comparisons were made between the relative combined paternity indices, powers of exclusion, accuracy, and turnaround time of the two methods.

Program Nr: 2335 from the 1999 ASHG Annual Meeting

Genetic Testing for Friedreich Ataxia. *M.J. Wick[1], V.L. Matthias Hagen[1], D.J. Allingham-Hawkins[2], M.A. Nance[3], N.T. Potter[4], for the Ataxia Molecular Diagnostic Testing Group[5]*. 1) Dept Lab Medicine & Pathology, Univ Minnesota, Minneapolis, MN; 2) Genetics Program, North York General Hospital, North York, Ontario, Canada; 3) Dept Neurosciences, Park Nicollet Clinic, St. Louis Park, MN; 4) Neurogenetics Laboratory, University of Tennessee Medical Center, Knoxville, TN; 5) Multiple Institutions.

The Ataxia Molecular Diagnostic Testing Group (AMDTG) was established to provide proficiency testing and outcomes data for the inherited ataxias and DRPLA. The AMDTG has previously presented data regarding proficiency testing for the autosomal dominant ataxias (SCA1-3, 6 and 7) and DRPLA (AJHG 1998;63:A239). Here we report data from the AMDTG proficiency testing for Friedreich Ataxia (FRDA). Of the 22 laboratories listed in GeneTests™ for clinical FRDA testing, 19(86%) agreed to participate in the study, and 15(68%) completed the proficiency testing. Each laboratory analyzed four specimens and responded to a questionnaire regarding methods and result interpretation. Inter-laboratory agreement of allele sizes was best for the sample with two expanded alleles, and expanded alleles were almost always distinguished from normal alleles, but normal and premutation sized alleles were not always reliably distinguished from each other. Differences in separation methods and molecular weight markers, and in (GAA)n repeat reference ranges used for result interpretation appear to be responsible for this variability. These results, which will be discussed by the AMDTG-associated laboratories, suggest the need to 1.) establish uniform repeat reference ranges, perhaps through a multi-center genotype/phenotype study, and 2.) standardize methods which allow for appropriate resolution and sizing of alleles with repeats ranging from 7 to over 1000 GAA repeats. (Supported in part by a grant from the National Ataxia Foundation).

Program Nr: 2336 from the 1999 ASHG Annual Meeting

Immunofluorescent staining for emerin in hair roots distinguishes Emery-Dreifuss muscular dystrophy patients and carriers from normal individuals. *G.R. Wilmot[1], S.T. Warren[2,3].* 1) Department of Neurology; 2) Departments of Biochemistry, Genetics, and Pediatrics, Emory University School of Medicine; 3) Howard Hughes Medical Institute.

Emery-Dreifuss Muscular Dystrophy (EDMD) is an X-linked inherited myopathy characterized by muscle weakness, joint contractures and cardiac conduction defects. The disease is caused by mutations in the EDMD gene which lead to a loss of the protein product emerin, which localizes to the inner nuclear membrane. The diagnosis can be established either by screening for EDMD mutations, of which over 70 are currently known, or by establishing the absence of emerin expression by immunofluorescent staining of tissue or less invasively by Western blot analysis of leukocytes. Although Western blots work well in EDMD patients where emerin is completely absent, they are less efficient for diagnosing carrier status where intermediate emerin levels are seen due to X-inactivation. We have therefore explored whether individual hair roots, which are clonally-derived following X-inactivation, can be immunostained for emerin in order to diagnose EDMD carriers and affected individuals. Hair roots (5-20 from each individual) were collected from normal control subjects (n= 4), genetically-confirmed EDMD patients (n= 4) and EDMD carriers (n= 3), fixed in acetone and processed for indirect immunofluorescence with a monoclonal antibody to emerin. Each root was scored as negative (no emerin staining), positive (>70% cells stained), or intermediate (1-70% cells stained). EDMD patients demonstrated negative staining in all roots (n=20), while normal controls were positive in 23 out of 24 roots. Of 30 total roots taken from EDMD carriers, 14 were negative, 6 were intermediate and 10 were positive, clearly distinguishing them from normal individuals. Thus, the analysis of emerin immunoreactivity in hair roots offers a convenient, efficient, and minimally-invasive method for diagnosing EDMD patients and carriers.

Program Nr: 2337 from the 1999 ASHG Annual Meeting

Detection of FMR1 Genotypes, Sex Chromosome Abnormalities, and Deletion Mutations by PCR Products With an automated DNA Sequencer. *M. Winkler, M. McGill, L. Jones, T. Tubman, H. Hamdan, R. Fenwick.* Quest Diagnostics, Inc., Nichols Institute, San Juan Capistrano, CA.

The molecular diagnosis of Fragile X syndrome is performed using PCR and Southern blot analyses to determine the size of the CGG repeat in the FMR1 gene. For the last two years we have used a non-radioactive PCR protocol to co-amplify the FMR1 CGG repeat and the CAG repeat in the androgen receptor gene from clinical samples. Fluorescently labeled PCR products are analyzed in an automated DNA sequencing system (ALFExpress). The size of each PCR product is determined relative to a set of molecular size standards using 'Fragment Manager TM' software (Hamdan et al., 1997, Molec. Diag. 4:259). The use of betaine in the PCR eliminates 'stuttering' often observed with amplification of triple repeats (Papp et al., 1996. Molec. Diag. 1:59). This improvement coupled with the resolution obtained with the automated sequencer allows us to define the FMR1 genotypes of both male and female individuals and has also allowed detection of X chromosome aneuploidies. Migration of the fluorescently labeled PCR products in the sequencing gel is detected after laser excitation and the results are presented as a chromatogram. Measurement of the number and heights or areas of the peaks allows discrimination of normal and abnormal alleles plus estimation of gene dosage. Through our normal assay we have been able to identify 47,XXX, 48,XXXY and 46,X (Turners syndrome) individuals. Two patients affected by Kleinfelters syndrome were also identified with this method, one of whom carried a Fragile X syndrome premuatation. We were also able to discriminate a FMR1 deletion mutation in a female hemizygous for the FMR1 gene. Detection of these abnormal alleles would have been very difficult if the conventional radioactive PCR and autoradiographic detection method had been used. This non-radioactive and semi-automated method is also conducive to improving the efficiency of the Fragile X syndrome assay since each diagnostic assay can accommodate 35 patients plus the appropriate controls.*FMR1*^{TM}.

Program Nr: 2338 from the 1999 ASHG Annual Meeting

Hemoglobin Alpha chain deletions may select for Factor V Leiden. *K. Yanamandra, D. Napper, H. Chen, D.W. Jalanivich III, T.F. Thurmon, M. Susla, M. Jeroudi, J.A. Bocchini, jr.* Dept Pediatrics, LSU Medical Ctr, Shreveport, LA.

Hemoglobin Bart's (Hb Bart's) is found in 5% to 12% of newborns. The amount may be as low as 1% when one alpha chain is inactivated (mostly in Africans) or as high as 25% when three are inactivated (mostly in Orientals). Factor V Leiden is usually caused by a G to A transistion at position 1691 in the Factor V gene. That results in an arg506gln amino acid residue change in the Factor V clotting factor protein, which renders it resistant to inactivation. The frequency of Factor V Leiden ranges from near 0 among Africans and Orientals to 15% in Greeks. *If Factor V Leiden is present in a locale,* frequencies have been found to be higher among persons selected for various types of thrombophilia or for frequent spontaneous abortions.

Our local population has an ethnic mix typical of US urban areas. In our newborn screening program, about 4% have Factor V Leiden, and about 14% of African-Americans have Hb Bart's. However, we found that, among newborns with Factor V Leiden, 28% have Hb Bart's. The alpha chain deletions that result in the presence of Hb Bart's *also change hematologic indices.* This may ameliorate prenatal thrombotic events stemming from Factor V Leiden, resulting in differential survival. We will present our figures to date, along with statistical analysis.

Program Nr: 2339 from the 1999 ASHG Annual Meeting

Negative Impact of Missed Appointments on Clinical Genetics Service Delivery. *A. Zimak, A. Hunter, L. Humphreys, M. Cappelli.* Genetics, Children's Hosp. Eas. Ontario, Ottawa, Ontario, Canada.

Patients who miss appointments or cancel with short notice cause significant disruption in the delivery of clinical genetic services, and they become relatively more important as the demands on limited genetic resources grow. Our purpose was to examine the problem of non-attendance at genetic clinics and to examine existing interventions used by genetic centres to improve the attendance rate. This study looked at non-attendance rates at 21 genetic centres across Canada. The participants were sorted into groups of: Small (<500 visits/yr; n = 8); Medium (500-2000 visits/yr; n = 7); Large(>2000 visits/yr; n = 6). All participants completed a survey concerning their no show rate for physician and genetic counsellor appointments; cancellation rates; perceived importance of their no show rate; and interventions used to improve attendance. The non-attendance rate for physician appointments at all of the centres was 6%, compared to 5.7% for counselling appointments. The cancellation rate was 12% for physicians and 7.5% for counsellors, and the total non-attendance and cancellation rate for all appointments was 10%. Sixty-five percent of the centres stated that non-attendance was a problem. Seventy-five percent of the centres were using three or more interventions such as reminder calls, letters to referring physicians, and notices of missed appointments, which had mixed results in reducing missed appointments. As a follow-up to these results, a second study using a patient-based questionnaire was conducted to examine reasons for non-attendance at a genetics clinic. The findings from both studies will be used to develop targeted interventions to reduce both no-show and late cancellations of appointments. Evaluating the outcomes of these interventions will likely be useful for other genetic and outpatient health centres experiencing similar problems.

Program Nr: 2340 from the 1999 ASHG Annual Meeting

The German Human Genome Project (DHGP). *J. Maurer.* Managing Office of the Scientific Coordinating Committee, German Human Genome Project, Heubnerweg 6, D-14095 Berlin, Germany.

The German Human Genome Project (DHGP) is a joint effort between the German Federal Ministry of Education and Research (BMBF) and the Deutsche Forschungsgemeinschaft (DFG). The scientific work started in 1996. Since then, the DHGP has had an increasing impact on the international activities of the Human Genome Project. The project is represented by a scientific coordinating committee (elected members: Rudi Balling, Munich; Hans Lehrach, Berlin; Jens Reich, Berlin). Projects cover large scale genetic linkage analysis with an emphasis on multifactorial and complex traits, physical mapping (focused on chromosomes 17, 21, and X), sequencing (particularly 7, 21, X, and full length cDNA), expression studies, bioinformatics, studies of model organisms (e.g. large scale ENU mouse mutagenesis screen), and human evolution. A major research interest is to reveal the function of genes, especially those with medical relevance. Currently the second phase of funding is about to begin. It is expected that the emphasis will be shifted to high throughput functional genomics. For a comprehensive listing of current projects please refer to http://www.dhgp.de/. Currently, more than 50 projects are funded that were selected by an internationally composed scientific advisory board. For the whole initiative about DM 45 Mio (roughly US$ 25 Mio) per year are available. To promote and channel the application of the obtained scientific results a patent and licensing agency was established that supports scientists in patent affairs (www.pst.fhg.de/pla/). The Resource Center (funded by BMBF) at the Max Planck Institute for Molecular Genetics, Berlin, and the Deutsches Krebsforschungszentrum, Heidelberg, constitutes the central structural unit. It generates, collects and files standardized reference materials and distributes them among all groups participating in the DHGP. Its extensive service can, however, also be obtained by all researchers world-wide (for information please refer to http://www.rzpd.de/).

Program Nr: 2341 from the 1999 ASHG Annual Meeting

Pericentromeric region of human chromosome 22: Boundary between duplication segments and gene-rich region. *H.E. McDermid[1], B. Roe[2], P. Brinkman-Mills[1], M.A. Riazi[1], L. Bridgland[1], S. Maier[1], G. Banting[1], L. Ray[2], T. Nguyen[2], S. Minoshima[3], N. Shimizu[3], T. Footz[1].* 1) Dept Biological Sci, Univ Alberta, Edmonton, AB, Canada; 2) Dept Chemistry & Biochemistry, Univ Oklahoma, Norman, OK; 3) Dept Molecular Biology, Keio Univ School of Medicine, Tokyo, Japan.

Cat eye syndrome (eye, anal, heart, kidney and facial defects and mental retardation) results from the presence of four copies of the pericentromeric 2 Mb of human chromosome 22q. Recent analyses of sequence from the pericentromeric regions of chromosomes 2 and 16 (Horvath et al, 1998) and 10 (Jackson et al, 1999) reveal a complex mosaic of duplication segments from elsewhere in the genome. Mapping of the pericentromeric region of chromosome 22 has indicated a similar pattern (Minoshima et al, 1998). We have constructed and sequenced a BAC/PAC contig spanning the second Mb of pericentromeric 22q. Sequence analysis of the proximal half of this contig reveals a transition from complex duplication segments to a unique gene-rich region. The duplication segments include the unprocessed pseudogene of the von Willebrand factor (F8VWFP) and a number of fragments of the kappa light chain immunoglobulin variable region. Also present are segments from chromosomes 2, 10, 11, 16, 21, X and Y, as well as a fragment of the lambda light chain immunoglobulin variable region duplicated from elsewhere on chromosome 22. Interspersed repeats found throughout this region show an unusual distribution which favours LINES over SINES, the opposite pattern of the more distal gene-rich region. The latter contains at least 9 chromosome 22-specific putative genes. These genes are present in four copies in cat eye syndrome and are therefore being investigated as candidate genes. However, the complex of repeats proximal to this gene-rich region contains many gene fragments duplicated from throughout the genome. This may result in the creation of new genes which could also be candidates for involvement in cat eye syndrome.

Program Nr: 2342 from the 1999 ASHG Annual Meeting

Physical and transcript mapping of human chromosome 14q32. *D.A. McFadyen, B. Kraus, L. Read, D.W. Cox.* Dept Medical Genetics, Univ Alberta, Edmonton, AB, Canada.

We are constructing a physical and transcript map of the most distal region of chromosome 14, and have sequence-ready material for the region. The 14q32 region contains genes for Usher syndrome, retinitis pigmentosa, and neuroblastoma. The interval of interest encompasses the cytogenetic band 14q32 and is flanked by the serine protease inhibitor gene cluster at the centromeric end, and by the immunoglobulin heavy chain (IGH) gene cluster at the telomeric end. By FISH mapping of markers throughout the region and physical data, we have estimated the total length to be approximately 7.8 Mb, considerably smaller than anticipated by linkage analysis. A YAC contig of 5.5 Mb extending from D14S81 (in the serine protease inhibitor gene cluster) to D14S293 has been constructed. The STS and EST content of the YAC contig distal to D14S78 and extending to D14S293, an estimated 1.8 Mb, has been used as a source of probes for the isolation of BAC and PAC clones from this region. The approximately 2.3 Mb region distal to D14S293 is underrepresented in YAC libraries and we have used linkage and radiation hybrid maps to develop probes for clone recovery. The region is currently represented by six contigs, consisting of 113 clones, from which bidirectional chromosome walking has been undertaken, using probes derived from the sequenced ends of clones, to close the gaps. The construction of a transcript map of 14q32 is being facilitated by the use of software that generates a non-redundant in silico library of ESTs that have been reported to map to this region of chromosome 14. Presently, 23 STSs and 36 ESTs have been placed on the contig. We have determined more precisely the order of some of the genes previously mapped to the region, including: EMAP, YY1, HSPCAL4, TNFAIP2, CDC42BPB, XRCC3, CKB, DNCL, AKT, DLK, JAG2, CRIP2, and ELK2. The comprehensive map that we are developing will assist in disease gene discovery in this region.

Program Nr: 2343 from the 1999 ASHG Annual Meeting

An Integrated Linkage-Radiation Hybrid Map of theCanine Genome. *C.S Mellersh[1], C. Hitte[2], M. Richman[1], F. Vignaux[2], C. Priat[2], S. Jouquand[2], P. Werner[3], C. Andr[2], S. DeRose[1], D.F. Patterson[3], E.A. Ostrander[1], F. Galibert[2].*
1) Clinical Research D4-100, Fred Hutchinson Cancer Res Ctr, Seattle, WA; 2) UPR 41 CNRS, Recombinaisons Gntiques, Facult de Mdecine, 35043 Rennes Cdex, France; 3) Section of Medical Genetics and Center for Comparative Medical Genetics, University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA 19104.

Purebred dogs are a unique resource for dissecting the molecular basis of simple and complex genetic diseases and traits. As a result of strong selection for physical and behavioral characteristics among the 300 established breeds, modern dogs are characterized by high levels of interbreed variation, complemented by significant intrabreed homogeneity. A high resolution map of the canine genome that contains both type I and type II markers is necessary to establish the syntenic relationship between the dog and other mammalian genomes and thus exploit the mapping power of this unusual resource. To this end we have constructed a radiation hybrid (RH) map of the canine genome and have integrated it with the most recent version of the canine linkage map. The map contains more than 600 markers, of which approxiately two thirds are microsatellites and one third are genes. The map likely identifies a syntenic group (a linkage group aligned with its corresponding RH group(s)) for each canine chromosome and is estimated to cover in excess of 95% of the genome. Partial alignment of the canine and human maps enables conservation of synteny between dog and human chromosomes to be investigated and also offers the opportunity for candidate gene studies suggested by initial linkages deduced from the primary linkage map.

Program Nr: 2344 from the 1999 ASHG Annual Meeting

Genomic sequencing and identification of 190 genes on human chromosome 22q11.1-q11.2. *S. Minoshima, T. Sasaki, K. Kawasaki, A. Shintani, K. Shibuya, S. Asakawa, N. Aoki, Y. Yoshizaki, J. Kudoh, N. Shimizu.* Dept Molecular Biol, Keio Univ Sch Medicine, Tokyo, Japan.

We have been analyzing genomic sequence of 3 regions on chromosome 22q11.1-q11.2. These include the proximal half of the cat eye syndrome (CES) region (D22S458-D22S50, 1.1 Mb), a 1.8-Mb contiguous region from Velo-Cardio-Facial syndrome (VCFS) "distal repeat" to immunoglobulin l gene locus (IGL) (BCRL2-GNAZ), and another contiguous 1.5-Mb region from BCR to the IGL-like gene (IGLL) region (BCR-TOP1P2). The total length of these regions is 4.4 Mb corresponding to 10% of the chromosome 22q. To date, we have completed a total of 3.6 Mb of genome sequencing for these regions. For the CES region covered with 4 BAC and 26 cosmid clones, 0.9 Mb has been processed. The sequence analysis predicted at least 7 genes, including neurofibromatosis type 1 (NF1)-like, g-glutamyl transpeptidase (GGT)-like, adrenoleukodystrophy (ALD)-like, CESK1, and von Willebrand factor-pseudogene (F8VWFP). For the IGL locus, a contiguous sequence of 1,025,415 bp was determined. The detailed analysis revealed 36 potentially active immunoglobulin V genes, 83 V pseudogenes, 7 J genes and 7 C genes. Thirteen non-immunoglobulin genes and pseudogenes were identified, including a gene belonging to phosphatase 2C family, topoisomerase 3b, VpreB, and melanoma antigen PRIME (Genome Res. 7: 250-261, 1997). For the VCFS distal repeat region, GGT.2, GGT-rel, BCRL2, POM121, the E2F factor 6 pseudogene, and a new gene which matches with 18 ESTs were found. For the IGLL region, a total of 25 genes were identified, including a suppressor gene for a malignant Rhabdoid tumor, hSNF5/INI1, both an active gene and a pseudogene for IGLL, glutathione S-transferase q (GSTT), 4 D-dopachrome tautomerase (DDCT), BCR, GGT, and GGT-related. GSTT and DDCT were found as a large cluster consisting of 9 GSTT and 4 DDCT genes/pseudogenes. A deletion of GSTT1 was found in one of 2 alleles. It is noteworthy that the GSTT1 deletion was homozygously found in 16% of the Caucasian and that it is associated with an increased rate for cancer.

Program Nr: 2345 from the 1999 ASHG Annual Meeting

Are common fragile sites preferential targets for the integration of mitochondrial DNA during evolution? *D. Mishmar[1], C. Kilger[2], T. Haaf[3], A. Rahat[1], S. Paabo[4], B. Kerem[1]*. 1) Genetics, Hebrew University, Jerusalem, Israel; 2) Lion Bioscience AG, Heidelberg, Germany; 3) Max Planck Institute for Molecular Genetics, Berlin, Germany; 4) Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany.

Fragile sites are specific unstable chromosomal loci characterized by gaps and constrictions on chromosomes from cells exposed to specific chemical conditions. In humans there are >100 fragile sites, classified as rare and common according to their prevalence in the population and mode of induction. Common fragile sites (cFS) are considered part of the normal chromosome structure and exhibit some characteristics of instability: gene amplification, chromosomal rearrangements and foreign DNA integration. Three cFS have been identified at the molecular level, all shown to be targets for viral DNA integration indicating that fragile sites are unstable genomic regions which tend to rearrange. Despite their instability, evolutionary conservation was found for several cFS, indicating that these sites might play an important biological role. The nuclear DNA of various organisms contains mitochondrial DNA (mtDNA) homologous sequences. These sequences are thought to arise by the integration of mtDNA into the nuclear genome during evolution. We have identified >90 mtDNA integration sites in the human genome by screening PAC libraries with mtDNA. Here we investigated the possibility that cFS might have been preferential targets for these integration events. We have studied four of the PAC clones which map to chromosomal regions (4q31, 3p22-23, 9q22, 13q31-32) known to harbor cFS (FRA4C, FRA3A, FRA9D and FRA13D, respectively). FISH analysis using these PAC clones was performed on metaphase chromosomes expressing cFS. Three of the clones (from 4q31, 3p22-23, 9q22) appeared to span a fragile site gap (FRA4C, FRA3A and FRA9D respectively), hence on different chromosomes from the same preparation their hybridization signals appeared centromeric, telomeric, or crossed the fragile region. These results suggest that mtDNA integration might have occurred preferentially at cFS. Thus, cFS might play a major role in chromosomal evolution.

Program Nr: 2346 from the 1999 ASHG Annual Meeting

Physical mapping of the candidate region for the gene causing Limb-Girdle Muscular Dystrophy type 2G (LGMD2G). *E.S. Moreira[1], T. Wiltshire[2], M. Vainzof[1], R.H. Reeves[3], M. Zatz[1], M.R. Passos-Bueno[1]*. 1) Depto. de Biologia, Instituto de Biociências, Universidade de São Paulo, Brasil; 2) Dept. of Psychiatry, University of Pennsylvania, Philadelphia, PA, USA; 3) Dept. of Physiology, Johns Hopkins University School of Medicine, Baltimore, MD, USA.

Limb-Girdle Muscular Dystrophy type 2G (LGMD2G), one of the 8 forms of Autosomal Recessive (AR) LGMD, was recently mapped by us to 17q11-12 through a genome-wide scan in one Brazilian family. Only 2 of 29 other Brazilian AR LGMD families tested with 17q11-12 markers are possibly linked to the LGMD2G locus, suggesting that this form of muscular dystrophy is rare in our population. The candidate interval for the LGMD2G gene, based on recombination analysis, was delimited between D17S1867 and D17S1814, which are estimated to be 3 cM apart. We are now constructing a BAC/PAC contig of this region. The clones have been selected either by screening of genomic DNA libraries or through scanning the Whitehead Institute web-site, using 28 STSs mapped to 17q11-12. Identification of overlaps between clones was performed using STS mapping and EcoRI fingerprinting. So far, we have been able to construct 3 contigs, including a minimum of 21 clones. Two of them are approximately 700kb and 500kb and harbor the proximal and distal flanking markers D17S1867 and D17S1814, respectively. The largest one, covering a region of about 900kb, comprises microsatellites D17S250, D17S1851, D17S946 and D17S1814, which are known to be non-recombinant in the LGMD2G families. Three novel microsatellites have been identified within the 900kb contig, all of them being polymorphic in our population. Genotyping of these markers in the LGMD2G families showed no recombination between any of them and the disease gene. In conclusion, the contigs are ordered as 700kb-900kb-500kb and the candidate region has a minimum of 900kb. Presently, we are conducting end sequence STS generation in order to close the remaining two gaps. Completion of this physical map will provide the framework necessary for the positional cloning of the LGMD2G gene. Supported by FAPESP, HHMI, CNPq, PRONEX.

Program Nr: 2347 from the 1999 ASHG Annual Meeting

Improving the Efficiency of Targeted Mutagenesis Using Bacteriophage l **Vectors.** *M.L. Mosaico, K. Woltjen, M.W.T. Unger, G. Bain, D.E. Rancourt.* Biochemistry & Molecular Biology, University of Calgary, Calgary, Canada, T2N 4N1.

Targeted mutagenesis is a pervasive biomedical technology that allows the engineering of specific mutations into the mouse genome. Its utility to human genetics is in the generation of animal models of disease and in the validation of drug targets. In the emerging functional genomics phase of the human genome project, it is anticipated that over 100,000 genes in the mammalian genome will need to be mutated. Following this agenda, we have initiated experiments to improve the efficiency of the targeted mutagenesis using bacteriophage l as a vector system. We have developed methods to streamline the construction of targeting vectors (TVs) by building them in bacteriophage l using phage-plasmid recombination. Using a recombination-proficient gene targeting phage, l2TK, we are able to introduce modification cassettes to desired sites using double-crossover recombination, thus obviating limitations in TV design, including restriction site availability. Using a novel mutagenesis method, custom mutations can be transferred from oligonucleotides to phage TVs using a plasmid interface. By developing a 129-ES cell genomic library in lTK, gene targeting phage can be rapidly isolated using recombination and modified as above. We have also explored whether intact bacteriophage may be used as a medium for introducing lTK TVs into ES cells. Typically TVs are introduced into ES cells by electroporation. While this is an effective method of delivery, it causes DNA shearing and can affect targeting efficiency either by reducing the total homology length or by severing the negative selection marker. We have electroporated TVs in phage capsids to potentially overcome these problems. We find that bacteriophage-mediated TV delivery yields targeted clones at efficiencies comparable to conventional methods. More importantly, we observe that two orders of magnitude less DNA-equivalents are required when using phage. These results suggest that phage-mediated TV delivery can significantly increase the absolute efficiency of gene targeting. As bacteriophage appear to be benign in ES cells, these observations indicate a possible future role for these vectors in human gene therapy.

Program Nr: 2348 from the 1999 ASHG Annual Meeting

A systematic approach for investigating primary iron overloads : Generation of iron-related SNPs. *J. Mosser[1], N. Soriano[1], F. Wojcik[1], V. Douabin[1], H. Ferran[1], S. Sachot[1], D. Lavenier[2], R. Moirand[3], Y. Deugnier[3], J-Y. Le Gall[1], V. David[1]*. 1) Faculty of Medicine, UPR 41 CNRS, RENNES, FRANCE; 2) IRISA, CNRS INRIA,Rennes, France; 3) Clinique des maladies du foie,CHU Pontchaillou, Rennes, France.

Hemochromatosis is essentially related to alteration of the HFE gene. In north european populations, the patients are mostly homozygous for the C282Y mutation but an incomplete penetrance of this mutation is suspected. Indeed systematic population screening studies showed that the frequency of the C282Y homozygotes was higher than that reported for the disease in the corresponding region. These discrepancies could be explained by the existence of modifier genetic factors. To identify these genes, we decided to use a candidate gene approach. As a first step of this study, we developped an informatic system to characterize putative SNP within genes involved in iron metabolism. This system provides a link between nucleotidic databases (UNIGENE clusters, Genbank ...) and EST trace data and then performs base calling and sequence assembly of all the sequences related to each gene of interest. The SNPs are selected when a mismatch of high-quality base calling occurs within a sequence identical to that of the assembling consensus. Among the 20 iron-related genes, 17 contained potential SNPs (average of 5 SNPs for each gene). Experimental SNP validation, performed by PCR-sequencing on 20 unrelated Breton control DNAs, revealed at most 50% reliability in SNP prediction. Further validations are required to define empirical threshold values for efficient SNP extractions. In parallel, we used both these validated SNPs and already known polymorphic markers to conduct systematic genotyping of families with C282Y incomplete penetrance. In the end, we expect that systematic genotyping will provide the characterization of some discriminative haplotypes in iron overloads or iron deficiencies.

Program Nr: 2349 from the 1999 ASHG Annual Meeting

***in silico* differential display.** *H. Murakami[1,2], T. Suzuki[2,3], J. Goto[1,2], S.-Y. Jeong[1,2], H. Hashida[1,2], N. Masuda[1,2], I. Kanazawa[1,2].* 1) Dept Neurology, Grad Sch Med, Univ Tokyo, Tokyo, Japan; 2) CREST, Japan Sci. and Tech. Copy., Tokyo, Japan; 3) Dept Medical Zoologyarasitology, Grad Sch Med, Nagoya City Univ.

Differential display (DD) is a powerful method to show the lineage-related gene expressions. We developed Computer programs predicting the result of the experiments of DD.
Analyzing and comparing with actual data, the band patterns of DD can increase the specificity of the result. The novel system of computer simulations for DD results is being used with the genetic database and some specific primers. We prefer to name it, "*in silico* Differential Display".;

The following are a list of components that the program is specifically designed to handle;.

1. Checking the cDNA database of a species in the Genbank and create a new database file to use *in silico* DD.
2. Adding the predicted PCR products in to the database file and make suitable landmarks for a band in a DD experiment.
3. Calculating the specific DD pattern by an input of the gene ID.

The band that is both observed in *in silico* DD and in the actual DD image are highly likely to be the area interest. If not, the unpredicted band in the actual DD is likely to be a novel gene.

In silico DD method has the advantage of having a low running cost and labor in comparison to the DNA array technique particularly. This method is likely to bring about greater efficiency for research involving the routine DD method.

Program Nr: 2350 from the 1999 ASHG Annual Meeting

Using the Invader™ Squared FRET Assay on a Panel of 24 Genomic Samples to Detect Single Base Changes at Multiple Loci. *B.P. Neri, D.M. Marshall, B. Aizenstein, E.L. Beaty, S.M. Law, K.W. Nichols, M. de Arruda.* Third Wave Technologies, Inc., Madison, WI.

As more of the human genome sequence is determined, a large number of single nucleotide polymorphisms (SNPs) are being discovered. It is becoming evident that SNPs are associated with certain medical disorders, response to drug therapies, and risk for some diseases. Therefore, the ability to detect SNPs in genomic DNA samples has important clinical and pharmacogenomic applications. The Invader™ Squared Assay employing FRET detection is a sensitive, low cost, and easy to use method that is capable of discriminating single base changes, insertions, and deletions directly on genomic DNA **without** the use of PCR. The components of the assay are two target specific oligonucleotides (termed the Invader and the signal probe), a FRET oligonucleotide, and the Cleavase® enzyme. The Invader and signal probe hybridize to the target, forming an overlapping complex only if the base of interest at the site of the SNP is present. This complex is a substrate for the structure specific Cleavase enzyme. The product of the cleavage event induces another cleavage event on the FRET probe which frees the signal fluorophore from the nearby quenching dye. Both reactions occur near the melting point of the oligonucleotides involved, so that multiple hybridization and cleavage events occur for each target molecule present, eliminating the need to amplify the target of interest. We will present data detecting single nucleotide changes at multiple loci on a panel of genomic DNA samples from 24 ethnically diverse individuals. All reactions involved the addition of less than 100 ng of genomic DNA (< 30,000 target molecules) and occurred in 96-well reaction plates that contained lyophilized reagents. Following a denaturation step, the reaction plates were incubated for 4 hours isothermally. The fluorescence signal was detected directly on the reaction plates without the need for any further sample manipulation. This demonstration shows that the Invader Assay is a versatile, simple, and low cost method that is suitable for the high throughput screening of SNPs.

Program Nr: 2351 from the 1999 ASHG Annual Meeting

Expression of non-deleted genes in the 18q- syndrome. *P. O'Connell[1], S.G. Hilsenbeck[2], J.D. Cody[3], Z. Wang[3], R.J. Leach[3]*. 1) Cell Biology, Baylor College of Medicine, Houston, TX; 2) Medicine, Baylor College of Medicine, Houston, TX; 3) Cellular and Structural Biology, University of Texas Health Science Center at San Antonio, San Antonio, TX.

The molecular mechanisms of disease pathogenesis in monosomic syndromes are poorly understood. Our recent studies of the 18q- monosomy indicate that while reduced gene expression of the deleted genes is common, some deleted genes can express at normal levels due to dosage compensation, and may influence the disease phenotype (Wang et al., Hum. Genet., in press). In the present study, we investigated whether alterations in the expression of non-deleted genes is also a feature of this disorder. This hypothesis was explored using two commercially available complementary DNA (cDNA)-based gene arrays interrogated with cDNA derived from poly-A+ RNA from either an 18q- patient or a normal control. The poly-A+ RNA was recovered from lymphoblastiod cell lines. The observed patterns of gene expression were subjected to statistical analysis utilizing a principle components-based approach, and genes known to be deleted in the 18q- patient exhibited appropriately reduced gene expression levels. Variations of gene expression between 18q- patients and normal controls were confirmed by a quantitative reverse-transcription polymerase chain reaction (RT-PCR) method tested on an expanded group of patients with 18q- syndrome and additional normal controls. These experiments indicate that altered gene expression of non-18q genes in 18q- patients was rare. A few genes (approximately 1%) showed up to two-fold increases or decreases in levels of gene expression in a subset of patients. These changes in gene expression in the non-deleted genes are of the same magnitude as seen for genes deleted in 18q- patients. These data support the notion that monosomic phenotypes can result from haploinsufficiency of deleted genes, but do not rule out a role for low magnitude variations of gene expression of certain non-deleted genes in some patients. These genetic variations may play a role in the variability of the phenotypic presentation of monosomies from patent to patient, even when their deletion breakpoints are similar.

Program Nr: 2352 from the 1999 ASHG Annual Meeting

Denaturing High Performance Liquid Chromatography (DHPLC) Detects *BRCA1* and *BRCA2* Mutations with High Sensitivity. *P. Oefner[1], D. Stoppa-Lyonnet[3], E. Fleischmann[2], D. Muhr[2], S. Pages[3], T. Sandberg[4], V. Caux[3], R. Moeslinger[2], G. Langbauer[2], A. Borg[4], T. Wagner[2]*. 1) Biochemistry, Univ Stanford, Palo Alto, CA; 2) Dept OB / GYN, Div Senology, Univ Vienna, Vienna, Austria; 3) Unite Genetique Oncologique, Inst Gustave Roussy, Villejuif, France; 4) Dept Oncology, Lund University, Lund, Sweden.

Denaturing high performance liquid chromatography (DHPLC) is a recently developed method of comparative sequencing based upon heteroduplex detection. To assess the sensitivity of this method 180 mutations in BRCA1 and BRCA2 were tested: First, we analyzed 131 different mutations (50 deletions, 9 insertions and 70 single base substitutions) that had been identified originally by DGGE, PTT, SSCP or direct sequencing. Second, 49 different mutations (46 single base substitutions, 2 deletions and 3 insertions) that had been reported previously were identified by DHPLC in the process of screening 183 HBOC families and 111 control individuals from world wide populations. Third, 30 index individuals with complete direct sequencing analysis of BRCA1 were reanalyzed by DHPLC. Fourth, 41 index individuals were concomitantly analyzed by both DGGE and DHPLC. All 180 different BRCA1 and BRCA2 mutations showed heterozygous DHPLC elution profiles. The reanalysis of 30 index individuals revealed 1 splice site mutation initially missed by direct sequencing. The concomitant analysis of 41 index cases showed that 4 out of 4 probably disease associated mutations were identified by DHPLC and 3 out of 4 by DGGE. We conclude that DHPLC is as sensitive as direct sequencing but significantly less expensive and less labor intensive.

Program Nr: 2353 from the 1999 ASHG Annual Meeting

An STS, EST/gene and YAC/PAC based physical map of the Split hand-Split foot type 3 (SHFM3) critical region on chromosome band 10q24. *R.S. Ozen[1], B.E. Baysal[2], F.E. Orkunoglu[1], B. Devlin[2], C.W. Richard III[3]*. 1) Dept Medical Genetics, Gulhane Med Academy Med Fac, Ankara, Turkey; 2) Department of Psychiatry, University of Pittsburgh Medical Center, Pittsburgh, PA; 3) Wyeth-Ayerst Research, Radnor, PA.

Split-hand/split foot malformation (SHFM, ectrodactyly) is a human limb malformation characterized by aberrant development of central digital rays with absence of fingers and toes, a deep median cleft and fusion of remaining digits. SHFM is clinically and genetically heterogeneous. Three SHFM disease loci have been genetically mapped to chromosomes 7q21 (SHFM1), Xq26 (SHFM2), and 10q24 (SHFM3). We have previously confined the SHFM3 locus to an approximate 2 Mb interval between D10S1147 and D10S1240 by analysis of a multigenerational Turkish family. We have also shown that SHFM3 displays transmission distortion. The mutant allele is preferentially transmitted from affected fathers to his offspring. The SHFM3 critical interval harbors other known disease loci including infantile onset spinocerebellar ataxia (IOSCA) and renal coloboma syndrome, which is caused by mutations in the PAX2 gene. To facilitate the identification of SHFM3, we have built a physical map across the critical region. We have analyzed and ordered 16 CEPH mega-YACs, which are selected based on the regional framework map from Whitehead Institute physical mapping database. Eight PAC clones were selected from previous physical mapping studies on the IOSCA locus. These clones are ordered by STS content mapping. A total of 39 STSs have been used to refine the physical map. Using the constructed map, we have further tested and mapped numerous ESTs and regionally localized known genes, including FGF8, PAX2, CYP17, HOX11 and LBX. We have localized FGF8, PAX2 and CYP17 and three anonymous ESTs (WI-3735, WI-6750 and WI-16946) within the SHF3 critical region. Mapping of additional transcribed sequences and further refinement of the map is underway. This physical map will help in the identification of SHFM3 and other disease loci on chromosome band 10q24, as well as in the elucidation of mechanism of transmission distortion.*SHFM1SHFM2SHFM3IOSCAPAX2*.

Program Nr: 2354 from the 1999 ASHG Annual Meeting

Optimizing full-length gene isolation by PCR based methods. *D. Patzak, M.S. Wehnert.* Institute of Human Genetics, Greifswald, Germany.

The structure of human genes can be detected from the genomic sequence and expressed sequences. Large-scale analysis of expressed clones (e. g. ESTs, SAGE) is often limited to partial sequences. Still the extension to of full-length expressed genes from pieces is a bottleneck for further functional analysis and disease association. Thus we tried to optimize the isolation of whole expressed genes from partial sequences. For this we used partial cDNA clones obtained from a human heart cDNA-library by reciprocal probing including the ribosomal protein L31 (as a control) and two unknown X-chromosomal cDNAs. The two unknown human cDNAs were used to screen the "I.M.A.G.E. consortium clone collection" by filter hybridization (RZPD Berlin and Heidelberg). No positive clones could be verified although EST entries (GenBank) indicate their presence. Spurred by some disadvantages of library screenings (e. g. missing signals, effort, incomplete cDNAs) we focussed on RACE-PCR (rapid amplification of cDNA ends). Using a commercial system (CLONTECH, Marathon-Ready cDNA) to amplify adaptor-ligated templates in both directions (5' and 3' extension) we could isolate differently spliced cDNAs in one case but only artifacts and parts of non-related genes in the other case. We developed modifications in order to overcome some disadvantages of the commercial RACE system (e. g. amplification of non-related genes, amplification of shorter ends). These modifications include: (i) adaptor-ligation to a PCR-product obtained from a specifically prepared full-length cDNA (ii) nuclease-digestion after the first cycle of PCR to prepare a more specific template for the following cycles and (iii) usage of end-primers (without adaptor-ligation) that preferentially work together with gene specific primers. Modification (i) and the combination of (ii) and (iii) were found to get the best results for specific amplification of 5' and 3' cDNA-ends.

Program Nr: 2355 from the 1999 ASHG Annual Meeting

Functional Mapping of the Human 17 Syntenic Region on Mouse Chromosome 11. *M.A Pershouse, W.W Cai, J. Klysik, W.M. Di, E.K. Brundage, A. Bradley, A.C. Chinault.* Molecular & Human Genetics, Baylor College of Medicine, Houston, TX.

Even with the completion of the sequencing of the human genome in the near future, there will exist functional data for only a small fraction of the 80,000 to 100,000 genes. The overall goal of this project is to provide functional evidence for genes mapping to human chromosome 17, a linkage group which is conserved on mouse chromosome 11. Our collaborators will employ phenotype-driven screens in mice or cell lines which have been manipulated by *loxP/Cre* chromosomal engineering techniques to contain large regions of segmental haploidy. These regions will be further targeted for ENU mutation to uncover recessive alleles. Our laboratory's role in this project is to establish a high resolution BAC map of this region. The map will be used for a number of purposes, but primarily to aid in finding the genes responsible for phenotypes seen in deletion or mutation mice. Mapping has been initially concentrated in the region from 56-66.5 cM to coordinate with initial deletion studies, but will eventually cover the entire 33-80 cM region. Mapping was accomplished by screening with labelled overgo primers designed against available STS markers and 3' and 5' UTRs. Gap closure was facilitated by BAC end sequencing, primer design, and rescreening of the library. Restriction digest fingerprints were used to optimize the rescreening process. To date, we have identified 299 BAC clones using 115 markers, with 605 total hits, or approximately 2 hits per BAC. We have also developed a high-throughput BAC prep which generates sequence-ready template, and have sequenced over 16 kb of BAC ends to date. Approximately 25% of our BAC end sequences map within known genes or to cDNAs within Unigene clusters, suggesting the usefulness of this data not only in probe generation, but in gap closure. Detailed maps of this region will be presented.

Program Nr: 2356 from the 1999 ASHG Annual Meeting

Comprehensive Transcript Analysis in Small Quantities of mRNA by SAGE-Lite. *D.G. Peters[1], A.B. Kassam[2], E. Heidrich-O'Hare[1], H. Yonas[2], R.E. Ferrell[1], A. Brufsky[3].* 1) Human Genetics, Univ Pittsburgh, Pittsburgh, PA; 2) Neurosurgery, Univ Pittsburgh, Pittsburgh, PA; 3) Hematology/Oncology, Univ Pittsburgh, Pittsburgh, PA.

Serial Analysis of Gene Expression (SAGE) is a powerful technique for global analysis of gene expression in a tissue or cell type of interest. Its chief advantage over other methods is that SAGE does not require prior knowledge of the genes of interest and provides quantitative and qualitative data of potentially every transcribed sequence in a cell or tissue type. Furthermore, SAGE can quantify low abundance transcripts and reliably detect small changes in transcript abundance between cell populations. However, SAGE demands high levels of mRNA which are often unavailable, particularly when studying human disease. To overcome this limitation, we have developed a modification of SAGE that allows detailed global analysis of gene expression in extremely small quantities of tissue. We have called this approach "SAGE-Lite". This technique was used for the global analysis of transcription in samples of normal and pathological human cerebrovasculature to study the molecular pathology of intracranial aneurysms (ICA). These samples, which are obtained during operative surgical repair, are typically no bigger than 1 or 2 mm^2 and yield <50 ng of total RNA. In addition, we show that SAGE-Lite allows simple and rapid isolation of long cDNA fragments from short (14 bp) SAGE sequence tags.

Program Nr: 2357 from the 1999 ASHG Annual Meeting

5' nuclease assay as a tool for SNP haplotype studies of cancer genes. *R.J. Peterson[1], V.J. Clark[1,3], M.W. Smith[2], M. Dean[1]*. 1) Laboratory of Genomic Diversity, NCI-FCRDC, Frederick, MD; 2) SAIC, Frederick, MD; 3) Graduate Program in Genetics, PSU, University Park, PA.

Genetic background influences a person's vulnerability to cancer. An important fraction of this background is functional variation due to Single Nucleotide Polymorphisms (SNPs). As part of the Cancer Genome Anatomy Project, the goal of this study is to investigate the effect of SNPs on the initiation and progression of cancer. Linkage disequilibrium analyses for a large number of candidate genes require scoring many SNPs. However, SNP analysis using traditional gel electrophoresis is slow and costly. Because of this, we have investigated the 5' nuclease assay (TaqMan, PE) as a way to rapidly query many SNPs. In this fluorescent method, discrimination occurs during PCR due to allele-specific probes that, when hybridized to template, are cleaved by the 5' nuclease activity of Taq polymerase. Cleavage results in increased emission of a reporter dye (VIC or FAM) that otherwise is quenched by the dye TAMRA. Eighteen SNP assays were designed using PE's Primer Express software and tested on the PE Prism 7700 Sequence Detection System. The genes queried were: *CCR2, CCR5, GPR15, SDF1, XRCC1, JAK3, CDH1, GSTP1, CYP17* and *ATM*. Sixteen of the eighteen SNP assays yielded excellent discrimination using a single set of reagent concentrations and thermal cycling parameters. Genotypes for SNPs in *CCR2, CCR5, GPR15* and *SDF1* were concordant with genotypes collected using restriction enzymes and SSCP. Consistent Mendelian transmission was observed in 40 3-generation CEPH pedigrees comprising 489 individuals. Allele frequencies were obtained in thirty each of Europeans, African-Americans, Hispanics, and Asians. Preliminary results indicate that cluster and discriminate analysis allow allele calling for data pooled across multiple plates. Association analyses are being performed for *XRCC1, JAK3, CDH1, GSTP1,* and *CYP17* in case/control cohorts for lung cancer (n=271), breast cancer (n=196), AIDS-related lymphoma (n=200), and AIDS-related Kaposi's sarcoma (n=200). The success rate reported here of the 5' nuclease assay indicates that it is a useful tool for haplotype studies.

Program Nr: 2358 from the 1999 ASHG Annual Meeting

Assessment of quantity and quality of DNA from formalin-fixed paraffin- embedded tissue using real time rapid cycle DNA amplification. *K. Pindolia[1], G. Pals[2], M.J. Worsham[1]*. 1) Cancer Genetics Research, Pathology, Henry Ford Health Systems, Detroit, MI; 2) Free Univeristy, Amsterdam, The Netherlands.

DNA analysis from archival tissue permits retrospective characterization of disease and has facilitated molecular data gathering from large population-based epidemiological study cohorts. Reliable quantification of paraffin DNA for PCR is problematic even with the more recent DNA extraction kits that provide better DNA quality and yield. We evaluated a real-time rapid cycle PCR approach to quantitate DNA from formalin-fixed paraffin tissue employing PCR of the beta-globulin gene using DNA-binding dye SYBR Green I or hybridization probes specific for the beta-globulin gene. The amount of paraffin DNA was measured as the number of copies of the beta-globulin gene. The specific hybridization probe for the beta-globulin gene was more reliable and accurate than PCR incorporating SYBR Green 1. Melting curve analysis permitted confirmation of the PCR product. DNA from formalin-fixed archival tissue can be reliably assessed for quality, quantity and confirmation of specific PCR products using real time PCR. This approach allows the use of more accurate sample dilutions to achieve optimal PCR.

Program Nr: 2359 from the 1999 ASHG Annual Meeting

The use of DNA microarray to study genes affecting a phenotype related with schizophrenia in a mouse model. *Y. Qiu, J. Cheng.* Genome Science Department, Lawrence Berkeley National Lab, Berkeley, CA.

It has been established that mice treated with the psychotomimetic drug phencyclidine (PCP or angel dust) mimic both positive and negative symptoms of schizophrenia. The schizophrenic phenotype in mice can be measured using the pre-pulse inhibition (PPI) of the startle response. We have shown that the PPI of C57bl/6J mice was reduced to ~50% of the normal level by administrating 10 mg/kg of PCP of acute dosage. The disrupted PPI in C57bl/6J mice was found to be restored by the appropriate dosage of antipsychotic drug clozapine. Clozapine alone did not change the PPI of the mice. A DNA microarray containing more than 3,000 mouse genes is used to assay the expression changes in the brain of mice induced by the drugs. Three specific brain regions (ventral midbrain, hippocampus, and cerebellum) have been assayed. The RNAs are extracted from each of the regions after each treatment of PCP, PCP+clozapine, or clozapine alone. The gene expression profiles are also followed at several time points after the drug treatments. The genes that are found to be consistently induced or suppressed by PCP but restored by clozapine are identified as candidate genes.

Program Nr: 2360 from the 1999 ASHG Annual Meeting

Denaturing High-performance Liquid Chromatography (dHPLC) Analysis of Pooled PCR Samples for High-throughput Screening for DNA Sequence Variants of Human Populations. *M. Radel, R. Aragon, C. Mazzanti, B. Kolachana, J. Vanakoski, J. Rudolph, D. Goldman.* Laboratory of Neurogenetics, NIAAA, NIH, Rockville, MD 20852.

Oefner and colleagues (1996) have detected DNA sequence variants using reverse phase HPLC in the presence of an ion-pairing agent. At the appropriate temperature, partially melted DNA heteroduplexes have relatively reduced affinity to the hydrophobic matrix, enabling DNA variants to be detected with high sensitivity. Here we report for the first systematic detection of single nucleotide polymorphisms (SNPs) in pooled PCRs from human genomic DNA samples and using the Eclipse HP dsDNA column in an 8.5 min run. Evaluation of dHPLC pooling was conducted in blind fashion, using one standardized dHPLC method, and a single temperature for variant in each DNA amplicon. The temperature for dHLPC variant detection was selected based on an initial temperature optimization conducted independently of observation of genetic variation. Three SNPs were evaluated: 5-HT5A , GABRA6 1031G>C and GABRA6 1236C>T and the amplicons were, respectively, 196, 233, and 365 bp in size. The individually amplified genomic DNAs from eight individuals chosen for their previously known genotype were pooled, melted and reannealed to form mixtures of homo- and heteroduplexes. For the three SNPs, detection was accurately accomplished at a level of one heterozygous individual mixed with seven homozygotes, in other words, one part (allele) in sixteen. An advantage of SNP detection in pooled DNAs is that the denatured rarer allele strands have a greater chance to reanneal to DNA strands corresponding to the more abundant allele, augmenting the heteroduplex DNA signal. Sample pooling conserves template DNA and enhances the efficiency of dHPLC for automated high-throughput detection of DNA variants.

Program Nr: 2361 from the 1999 ASHG Annual Meeting

MGD: A Comparative Mapping Resource. *S. Ramachandran, J.A. Blake, J.A. Kadin, J.E. Richardson, M.T. Davisson, J.T. Eppig, and the Mouse Genome Informatics Group.* The Jackson Laboratory, Bar Harbor, ME.

An extensive catalogue of genes will be available when the Human Genome Project is completed. But information pertaining to gene function, genome organization and evolution will not be obtained immediately. Studying similar genes in different organisms can generate this information. Comparative maps provide data on gene neighbors, gene environment and evolution, thus allowing greater insight into the wider processes of genetics and disease. Candidate disease loci and complex disease trait information can sometimes be transferred across species and aid in the development of animal models of human disease.

The Mouse Genome Database (MGD) is a community resource of mouse genetic and biological information. MGD includes a scientifically curated data set designed to provide the scientific community with access to comparative genomic data. Homology assertions are extracted from literature, Chromosome Committee reports and personal communications and integrated into MGD where they can be accessed by the user.

Information can be retrieved from MGD in several ways. The web interface allows users to query the database using a simple, flexible form. A concise homology assertion table listing species, gene symbols, chromosomal assignments, supporting references and hypertext links to online species databases comprises the query result. Several different comparative map displays can also be generated. Comparative maps can be displayed directly on a web browser or created as postscript files suitable for printing. Oxford Grid displays are also available as a genome-wide graphical display tool. The user can thus visualize evolutionarily conserved regions between two species.

MGD encourages community submissions of data supporting novel homology relationships and updates to existing information.

http://www.informatics.jax.org

Supported by NIH Grant HG00330.

Program Nr: 2362 from the 1999 ASHG Annual Meeting

Mapping and characterisation of an X-linked mental retardation locus (MRX) within Xp11.2. *F.L Raymond[1], J. Bonnington[1], S. Clegg[2], L. Willatt[1], D. Trump[1].* 1) Medical Genetics, Cambridge Institute for Medical Research, Cambridge University, Cambridge, UK; 2) Sanger Centre, Hinxton, Cambridge, UK.

There are over 60 families reported with non-syndromic X linked mental retardation. No gene in Xp11.2 has yet been identified that causes non-specific mental retardation although linkage to this region is described. We present 2 cases of MRX one with a familial interstitial deletion of Xp11.2 and the other with a de novo X autosome translocation with the X breakpoint at Xp11.2. The familial interstitial deletion was ascertained through a boy who presented with severe developmental delay. His mother also had learning difficulties but was less severely affected, although they both had similar behavioural phenotypes. The deletion was identified on G-banding and has been further defined by fluorescent in situ hybridisation using PACs from the region (http://www.sanger.ac.uk). The breakpoints lie between PACs dJ154P24(telomeric) and dJ1158B12(centromeric). Mapping of the boundaries of the deletion using further PACs, BACs and STS markers over this region has narrowed the candidate interval for defining a further MRX gene. The de novo translocation was found in an adult with profound mental retardation, unable to live independently. G banding analysis identified the X chromosome breakpoint to be also within Xp11.2. Fine mapping by fluorescent in situ hybridisation with PACs locates the breakpoint telomeric to the first case and lies between dJ8N8(telomeric) and dJ154P24(centromeric). This suggests there are at least 2 locii for MRX within Xp11.2 which lie on either side of dJ154P24.

Program Nr: 2363 from the 1999 ASHG Annual Meeting

Updates to VariationView: an applet to record and manage variation data, and its application to the tuberous sclerosis genes. *M.P. Reeve[1], V. Whittemore[2], D. Kwiatkowski[3].* 1) Genetics & Bioinformatics, Walter & Eliza Hall Institute, Parkville, Victoria, Australia; 2) National Tuberous Sclerosis Association, Landover, MD; 3) Genetics Laboratory, Hematology Division, Brigham & Women's Hospital, Boston, MA.

The VariationView java applet provides an intuitive graphical interface for entering insertions, deletions, and single-base changes in genomic and coding DNA context. The user can view the spectrum of variations from the exon or sequence level and by clicking on a variation see the effect of the change on the DNA and coding level. Variations are input by selecting the base(s) in question and the index and correct nomenclature are automatically.

In the process of collecting the mutations and polymorphisms for the tuberous sclerosis genes TSC1 and TSC2 a number of updates have been made to VariationView. These include new ways of filtering data for viewing, expanded storage of clinical information, an exon overview of variations by effect on coding sequence, inclusion of polymorphisms, and new output and printing features. An overview of TSC1 and TSC2 published variations will also be presented.

The VariationView should be generically applicable to other variation projects. As input it can take coding, genomic DNA, or a combination of the two, and existing variations can be loaded in batch format. Data is stored in MiniSQL, a light-weight relational database, and accessed from the applet through JDBC.

VariationView has been developed with the support of the National Tuberous Sclerosis Association and is currently being used as a repository for the published mutations in TSC1 and TSC2. The database can be viewed at http://zk.bwh.harvard.edu/ts.

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Program Nr: 2364 from the 1999 ASHG Annual Meeting

Refined mapping of human chromosomal region 9p22 distal to the interferon gene cluster and construction of a transcript map of 1.2 Mb surrounding RP S6 gene. *A. Reigo[1,2], T. Lushnikova[1], I. Laanpere[1,2], R. Ilisson[1], A. Metspalu[1,2]*. 1) Dept.of Biotechnology, Tartu University Institute of Molecular and Cell Biology, Tartu, Estonia; 2) Estonian Biocentre, Tartu, Estonia.

The area telomeric of interferon gene cluster on human chromosome 9p has been shown to be involved in tumor growth regulation by several authors. Aiming to find new disease-related genes in this region, we have constructed an undisrupted double-linked 4.5 Mb YAC contig (12 clones) between D9S2016 (IFNB)and D9S157, based on restriction analysis with 10 rare-cutting enzymes, end-clone hybridisation and polymorphic marker PCR. The refined order of 13 markers included in the contig differs from published data (Bouzyk 1997; Kenmochi 1998). 10 YACs connecting the two above-mentioned well-known chromosomal landmarks were screened for the presence of 18 anonymous DNA markers selected from databases (radiation hybrids of the best likelihood) and for 17 newly-generated markers, increasing the mean density of reliable markers in this area to 8 per 1 Mb. Current stage of our project focuses on expression mapping of a 1.2 Mb subregion covered by RP S6-containing YAC clone 804B9, which was used as a probe for screening a chromosome 9 specific cosmid library and also for direct cDNA selection from a human fetal brain cDNA library. The localisation of this YAC clone on 9p22 was confirmed by FISH. 86 cosmid clones from the primary selection have been organized into a full length coverage contig with 3 minimal gaps according to their restriction fragment and L1-fingerprints. Analysis of 52 positive cDNA clones (end-sequencing, database similarity search, dot-blot and Southern hybridisation to the selected cosmid clones) has revealed 14 cDNAs of interest. 6 of them (one with no highly similar matches in database, one 94% similar to neurodegeneration-related protein mRNA, two including protein kinase domains and two similar to anonymous ESTs) are subjects to tissue expression analysis as most promising to represent new genes in this functionally interesting human chromosomal region.

Program Nr: 2365 from the 1999 ASHG Annual Meeting

Upstream cloning methods applied to intronic sequence: cloning the insertion point of the partial duplication of the a**-7 nicotinic cholinergic receptor subunit (CHRNA7) gene on chromosome 15q13-q14.** *B.P. Riley[1], A.M. Makoff[1], M. Mogudi-Carter[2], T.J. Jenkins[3], R. Williamson[4], D.A. Collier[1], R.M. Murray[1]*. 1) Dept. of Psychological Medicine, Inst Psychiatry, London, England; 2) Dept. of Psychiatry, Baragwanath Hospital, Soweto, South Africa; 3) Dept. of Human Genetics, University of the Witwatersrand, Johannesburg, South Africa; 4) The Murdoch Institute, Royal Childrens' Hospital, Melbourne, Australia.

Recent reports have strongly linked the a-7 nicotinic cholinergic receptor gene (CHRNA7) on chromosome 15q13-q14 to a sensory gating deficit common in schizophrenics, and have shown suggestive results linking this receptor gene to the primary phenotype of schizophrenia in two samples of North American families. We have previously shown suggestive results for linkage to schizophrenia using the same map of markers as the original report in South African Bantu families. A partial duplication of exons 7 to 10 with at least four novel upstream exons has been identified in the same chromosome region. In order to generate further markers in the region and to test for variability in the duplication, we wished to clone the insertion point of the duplication. Promoter cloning methods provide an efficient alternative to subcloning large genomic clones for upstream walking. Using the Genome Walker upstream promoter cloning kit with two DNA polymerases and nested primers in known sequence at the 5' end of exon 5, we generated a set of 21 fragments from ten reactions varying from 0.4 to 3 kilobases (Kb) in length. Nine of these fragments were ligated into pGEM directly from the reaction mixture. Three larger fragments (1.4-3 Kb) required gel purification before ligation. Sequencing of these fragments reveals two species, one with an uninterrupted, and one with an interrupted, Alu repeat sequence. All clones are positive for either known exon 5 sequence or for intron 4 sequences previously obtained by direct sequencing of two P1 clones positive for exons 5-10 of CHRNA7. An assay for the breakpoint is currently being developed.

Program Nr: 2366 from the 1999 ASHG Annual Meeting

GENINTER/GENAROM, a new version of GENATLAS to represent and simulate genetic and molecular interactions. *M. Roux-Rouquie[1], C. Capponi[2], M. Page[3], C. Mugnier[1], M-L. Chauvet[1], J. Frezal[1]*. 1) Genetique Medicale U393, INSERM Enfants-Malades, Paris Cedex 15, France; 2) Laboratoire dInformatique de Marseille, UP, France; 3) INRIA Rhne-Alpes UPMF Grenoble France.

GENAROM, the knowledge base dedicated to the representation and simulation of genetic and molecular interactions, is currently under development using a new knowledge representation (KR) system called AROM. AROM introduces novel features in the world of KR systems, two of which are especially important in the context of interactions. Firstly, AROM (acronym of Associating Relations and Objects for Modeling) deals with relations as well as objects. Relations are very powerful for representing knowledge in domains where objects are highly interconnected, as it is the case in biology for interactions. Secondly, AROM uses an explicit model of time, allowing one to model and simulate dynamic systems like interactions networks, a task currently out of the scope of existing KR systems. In addition, AROM allows one to categorize objects (genes and/or products) as well as relations among these objects. As a first step to construct GENAROM, we are involved in concept identification and vocabulary characterization, and currently two kinds of ontologies are under development, which refer to structures and processes. The internal structure of these ontologies consist of tree-like conceptual hierarchies with multiple inheritance (composition and specialization). Once ontologies have been identified and represented in the knowledge base GENAROM, they will be used as a basis for the categorization of new structures and processes. AROM does provide us with interactive mechanisms which are of great help to classify some new knowledge among the identified classes and relationships of ontologies. Automatic programs are planned to transfer the knowledge represented in GENAROM toward data represented in GENINTER of which structure was deduced from GENAROM. Referring to ontologies, biological functions will be modeled and represented, mainly as relationships among structures and/or processes.

Program Nr: 2367 from the 1999 ASHG Annual Meeting

Physical and transcript map of CCM1 candidate interval on chromosome 7q. *T. Sahoo[1], J.W. Thomas[2], S.-Q. Lee-Lin[2], P.M. Kuehl[2], C. Dokken[3], E.W. Johnson[3], E.D. Green[2], D.A. Marchuk[1]*. 1) Dept Genetics, Duke Univ Medical Ctr, Durham, NC; 2) NHGRI, NIH, Bethesda, MD; 3) Barrow Neurological Institute, Phoenix, AZ.

Cerebral cavernous malformations (CCM) are congenital vascular anomalies of the brain responsible for significant neurologic disability. Autosomal dominant forms of CCM have been described, and we and others have shown that a gene for CCM (CCM1) maps to chromosome 7q. The current CCM1 candidate interval encompassing a 1.9 Mb region on Chr 7q as defined by a common hispanic haplotype and additional recombinants has been completely sequenced. This has enabled us to construct a complete physical map of the CCM1 interval. We are using a sequence-based strategy to identify candidate genes. This involves computational analysis of the sequence using BLAST to search for known genes/transcripts, as well as the gene prediction programs GRAIL II, GENESCAN, and MZEF to identify putative coding regions within raw genomic sequence. Exons thus identified by computer analysis of genomic sequence are being amplified from our panel of patient genomic DNAs and subsequently sequenced to identify authentic mutations. We have so far identified and analyzed approximately 200 putative exons effectively covering about 960 Kb of genomic sequence. A number of known genes (CDK6, KRIT1, AKAP450, YOTIAO, PFTAIRE) that we have identified as mapping within the CCM1 interval are also being analyzed at the genomic sequence level. We have not yet identified any mutations in the CDK6 and AKAP450 genes. We are also including a strategy to detect micro- or macro-deletions that may be present in this region of Chr7q in CCM1 patients using somatic cell hybrids generated using isolated affected or unaffected Chr 7 from a number of our patients. Identification of the CCM1 gene and the nature of the mutations will be the first step towards achieving a better understanding of the pathogenesis of this disorder.

Program Nr: 2368 from the 1999 ASHG Annual Meeting

Automated method to construct high-throughput BAC physical maps. *J. Sainz, S. Gudjonsson, O. Gustafsson, H. Magnusson, G. Arnason, K. Benediktsson, G. Ludviksson, K. Stefansson, J. Gulcher*. Decode Genetics, Reykjavik, Iceland.

We have developed a highly automated method to rapidly construct large physical maps of BAC clones. At present we are constructing human BAC maps, spanning up to 40-Mb regions with a 0.1 Mb average density of markers, in less than 10 working days/person. The method is based on a two-step hybridization approach of pooled oligomers of genetic markers against a RPCI-11 BAC library with 12X genomic coverage robotically gridded in 6x6 density. Prior to the hybridizations, the primers are analyzed for repeat, E. coli and vector sequence content, and discarded according to the percentage and/or the melting temperature of the homology. The whole sequence analysis process is performed automatically by a computer program. The first hybridization step is based in two pools of primers, forward and reverse, and only the coincident signals in both hybridizations are selected as positive clones. This approach is very robust and, according to our data, has reduced the number of false positives below 5%. In the second step, the primers are pooled in rows and columns or in a binary approach and hybridized against the re-arrayed and gridded positive clones from the first step. Reading of positives is performed in both steps automatically by a computer program from scans of autoradiograms or phosphorimager files. The results provided by the computer program can be edited manually, if necessary, to improve the quality of the readings. Analysis of data to provide marker/BAC assignments and a graphical display of the physical map is also computerized. BAC/markers associations are confirmed by PCR and all the information is stored in a database that can detect clones contained in more than one chromosomal region as a quality control. The gaps are closed using the same method with new markers obtained from the BAC-ends or from data-mined sequences.

Program Nr: 2369 from the 1999 ASHG Annual Meeting

Localizing, ordering and orienting mouse collagen genes by high resolution FISH. *R. Sallinen[1], A.-P. Kvist[2], A. Latvanlehto[2], M. Rehn[2], I. Eerola[3], M.-L. Chu[4], P. Bonaldo[5], G.M. Bressan[5], B. Saitta[4], T. Pihlajaniemi[2], E. Vuorio[3], A. Palotie[1], M. Wessman[1], N. Horelli-Kuitunen[1].* 1) Departments of Clinical Chemistry and Biosciences, University of Helsinki, Helsinki, Finland; 2) Department of Medical Biochemistry, University of Oulu, Oulu, Finland; 3) Department of Medical Biochemistry and Molecular Biology, University of Turku, Turku, Finland; 4) Thomas Jefferson University, Philadelphia, USA; 5) Institute of Histology and Embryology, University of Padova, Padova, Italy.

High resolution fluorescence *in situ* hybridization (FISH) enables accurate ordering and distance measurements as well as determination of transcriptional orientation of genes. So far, high resolution FISH has mostly been used in human genome mapping but was here applied to the mouse genome.

We have used mouse metaphase chromosomes, mechanically stretched chromosomes and free DNA fibers for localizing, ordering and orienting five collagen genes by FISH. This was the first time mechanically stretched mouse chromosomes were used for physical mapping. Using metaphase FISH *Col6a1*, *Col6a2* and *Col18a1* were localized to mouse chromosome 10B5-C1, *Col13a1* to 10B4 and *Col10a1* to 10B1-B3 confirming the earlier linkage results. These regions are known to be homologous between human and mouse chromosomes. Mechanically stretched mouse chromosomes were used to determine the order of these five collagen genes and also to determine the transcriptional orientation of *Col13a1* and *Col18a1*. The most likely order of these genes on chromosome 10 is cen-*Col10a1*-*Col13a1*-*Col6a2*-*Col6a1*-*Col18a1*-tel. The transcriptional orientation of *Col6a1*, *Col6a2*, *Col13a1* and *Col18a1* is head to tail from telomere to centromere.

The comparison of the physical mapping results of human and mouse homologous collagen genes demonstrated that these five genes are clustered in mouse unlike in human. Chromosomal rearrangements and coincidence could together be the reason for clustering of these collagen genes in mouse. *Col6a1*, *Col6a2* and *Col18a1* might also share some transcriptional elements based on the short distance separating them.

Program Nr: 2370 from the 1999 ASHG Annual Meeting

High-density EST map of the rat. *T.E. Scheetz[1], M. Raymond[1], J. Zhang[1], A.M. McClain[1], C.A. Roberts[3], D.Y. Nishimura[1], T.L. Casavant[3], M.B. Soares[1,2], V.C. Sheffield[1]*. 1) Pediatrics and HHMI, University of Iowa, Iowa City, IA; 2) Physiology, University of Iowa, Iowa City, IA; 3) Electrical and Computer Engineering, University of Iowa, Iowa City, IA.

We are in the process of generating a high density EST map of the rat, placed on a framework of genetic markers. We have generated duplicate radiation hybrid mapping data on nearly 9000 nonredundant ESTs identified by our Rat Gene Discovery and Mapping Project (http://ratEST.uiowa.edu). We are placing these ESTs on published framework maps of genetic markers (1,2) as well as maps being constructed locally. Our ability to maintain a rate of novel EST discovery relies upon the method of serial subtraction of pooled, normalized 3' cDNA libraries (3). To date, we have been able to sustain a novelty rate of over 45% across more than 59,000 3' end reads, yielding over 27,000 novel clusters of 3' EST sequences.

To date, we have mapped, in duplicate, nearly 9000 novel rat ESTs, and have placed over 7000 of those ESTs on our frameworks maps. The chromosome framework maps have an aggregate total size of 11,000 cR, yielding an average physical correspondance of 270 kb per cM. Duplicate scores were used to create a consensus vector for every EST mapped. Discordant vectors were not utilized. The frameworks were built, and the placement maps created, using the RHMAPPER software package from MIT. Genetic marker order derived in the frameworks was compared with existing genetic maps available from the Whitehead Institute.

This EST map of the rat will be a valuable resource for constructing human-rat and mouse-rat syntenic maps, as well as aiding in the positional cloning of genes for rat models of human diseases. A web-based interface to the maps is available at http://ratEST.uiowa.edu/rat-map.

(1) Steen, R.G., et. al., Genome Research, Vol. 9, 1999, pp. 1046R-1046R.
(2) Watanabe, T.K., et. al., Nature Genetics, Vol. 22, Num. 1, May 1999, pp. 27-36.
(3) Bonaldo, M.F., Lennon, G., and Soares, M.B., Genome Research, Vol. 6, Num. 9, 1996, pp. 791-806.

Program Nr: 2371 from the 1999 ASHG Annual Meeting

Comparative transcriptional mapping of human chromosome 20q13 segment and identification of new genes as candidate for imprinting. *D. Schlote[1], S. Kussmann[1], A. Hehr[1], K. Koerber[1], H. Miller[2], J. Peters[2], I. Hansmann[1].* 1) Institut fuer Humangenetik, Universitaet Halle, Halle/Saale, FRG; 2) Medical Research Council, Harwell, UK.

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 lethalities 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 chromosomal region. Two mouse models have been generated carrying reciprocal translocations (T1Goe and T30H) which served to define chromosomal segments of the parental source effect. Based on two contigs (YAC, P1) covering a 2Mb imprinting region 1 (IR1) and a 5Mb imprinting region 2 (IR2), we have assigned several human ESTs into this chromosomal segment, by means of Southern hybridization to PFGE blots of mouse YACs. These EST markers were used to screen a human BAC library by PCR. Altogether 17 BACs have been arranged into a preliminary map of 550 kb in length for the 20q13 region suspected to carry imprinted genes. Successful sequencing of isolated BAC inserts representing genomic DNA of this chromosomal region results in defining further novel STS sites in this segment. To investigate the expression pattern multiple tissue Northern blot analysis has been done as well as RT-PCR of all assigned ESTs classified as unidentified transcripts by the means of sequence homologies. Based on these results we have already isolated several complete cDNA clones mapping in the region of interest using a marathon ready cDNA library and a PCR screenable cDNA library (RZPD, Berlin). Subfragments of the BAC clones carrying these sequences are being isolated for cloning the corresponding human gene which would serve as candidates to search for their monoallelic expression in mouse and man as well as their potential association with a given human disease. This work was supported by a grant of the DHGP.

Program Nr: 2372 from the 1999 ASHG Annual Meeting

Genomic Structure of the Human Machado-Joseph Disease (MJD/SCA3) Locus on Chromosome 14q32.1. *C. Sell, C. Cemal, S. Chamberlain.* Molecular Genetics, Imperial College of Science, Technology and Medicine, London, UK.

Machado-Joseph disease (MJD) or spinocerebellar ataxia 3 (SCA3) is an autosomal dominantly-inherited neurodegenerative disorder caused by pathological expansion of a (CAG)n repeat motif present within the coding sequence of the MJD1 gene. We report here the genomic organisation of the human disease locus, determined as the consequence of the strategy to generate a representative mouse model of the human disease using YAC transgenesis. In the original description of the disease gene, subdivision of the sequence into four exons was reported . We now demonstrate that the putative exon 1 and 3 sequences actually comprise three and two exons respectively, with the CAG repeat motif now located in exon 7. Exonic sizes range from 45 to 869 bp. Exon-intron structure and primer sequences designed to facilitate full characterisation of each exon are described. It is possible that the latter may be useful for systematic screening of individuals with sporadic cerebellar ataxia for conventional non-triplet repeat expansion mutations present within the gene sequence.

Program Nr: 2373 from the 1999 ASHG Annual Meeting

Physical and transcript mapping of the *CLN6* critcal region on chromosome 15q22-23. *J.D. Sharp[1], R.B. Wheeler[1], R.A. Schultz[2], J.M. Joslin[2], B.D. Lake[3], M. Fox[4], S.E. Mole[1], R.E. Williams[1], R.M. Gardiner[1]*. 1) Dept. of Paediatrics, Royal Free and University College Medical School, UCL, London, UK; 2) Eugene McDermott Center for Human Growth and Development, UTSWMC, Dallas, USA; 3) Dept. of Histopathology, Great Ormond Street Hospital for Sick Children, London, UK; 4) MRC Human Biochemical Genetics Unit, UCL, London, UK.

The neuronal ceroid lipofuscinoses (NCLs) are a group of progressive neurodegenerative disorders characterised by visual failure, epilepsy and the accumulation of an autofluorescent lipopigment in neurones and other cells.

The *CLN6* gene codes for a variant form of late infantile NCL and has been mapped to chromosome 15q22-23 by homozygosity mapping. Using a YAC and PAC contig the critical region was narrowed to approximately 200 Kb spanned by two PACs. These have been sequenced as part of the chromosome 15 sequencing project at UTSWMC. The sequence is being analysed using traditional gene identification methods including cDNA library screening and 5' RACE. In addition we are also using a range of gene prediction programmes from the NIX database at HGMP (http://www.hgmp.mrc.ac.uk/NIX/) including GENSCAN, MZEF, GRAIL-2. Two known genes, *MEK5* and *hnRNP* core protein A1 have been identified in the region. In addition at least two novel transcripts are present within the critical region and are being analysed as candidates for *CLN6*.

Program Nr: 2374 from the 1999 ASHG Annual Meeting

Gene Ontology: a controlled vocabulary to describe the function, biological process and cellular location of gene products in genome databases. *D.R. Shaw[1], M. Ashburner[2], J.A. Blake[1], R.M. Baldarelli[1], D. Botstein[3], A.P. Davis[1], J.M. Cherry[3], S. Lewis[4], C.M. Lutz[1], J.E. Richardson[1], J.T. Eppig[1].* 1) Mouse Genome Database, The Jackson Laboratory, Bar Harbor, ME; 2) FlyBase/European Bioinformatics Institute, Hinxton, Cambridge, UK; 3) Saccharomyces Genome Database, Dept. of Genetics, Stanford University, Stanford, CA; 4) Berkeley Drosophila Genome Project/FlyBase, Berkeley, CA.

The Gene Ontology (GO) project is a collaboration between research groups at three model organism databases (see institutions, above). The goals are to develop controlled vocabularies to describe the functions, biological processes and cellular locations of gene products and to annotate genes in the respective databases. A gene product may be one or more RNAs or proteins, and each of these may have more than one function, and could be localized to more than one cellular component. For mouse and human, gene nomenclature has been used frequently to describe gene products. While the gene name should be a unique designation, trying to attach significant biological information to the name can be problematic. Through the use of GO terms to associate a gene's products with its functions, multiple relationships can be captured and new or more precise functions can be added as more is learned. The GO vocabulary contains functional descriptions including broad terms such as "enzyme" or "aminosugar metabolism" and more specific terms such as "glucosamine catabolism" or "Toll receptor ligand." Over 5000 terms have been added to the GO vocabulary, though it is far from complete. Definitions of terms are included. A hierarchical list of the terms is available online at http://www.ebi.ac.uk/~ashburn/GO/. We hope that this controlled vocabulary will be adopted by other genome databases and become a community-wide effort. The GO vocabulary and its associated search tools should facilitate complex cross-species functional queries across the contributing databases. The Mouse Genome Database, http://www.informatics.jax.org/, has begun supplementing its gene descriptions with the GO vocabulary. MGD is supported by NIH grant HG00330.

Program Nr: 2375 from the 1999 ASHG Annual Meeting

Measurements of relative expression levels on microarrays using gene copy numbers as internal reference. *J. Shi, D. Che, P. Bao, U. Müller.* Advanced Technology, Vysis, Inc., Downers Grove, IL.

Determination of relative gene copy number differences between differentially labeled sample DNA and reference DNA is the basis for Comparative Genomic Hybridization and has been demonstrated to work equally well in formats employing DNA microarrays. A similar approach has been used for array based expression analyses, whereby differentially labeled cDNAs are co-hybridized to the chip. We have developed an assay format by which differentially labeled cDNA from a test tissue is co-hybridized with genomic DNA (gDNA) from the same test tissue and the gDNA from a reference tissue. The assay involves extraction of both genomic DNA and mRNA from the same cell line or tissue. The gDNA was labeled with SpectrumGreen-C-4-dCTP (Vysis), and the mRNA was converted to cDNA by reverse transcription in the presence of Cy-5-dCTP (Amersham). Competitive simultaneous hybridization of gDNA, cDNA, total human reference DNA (labeled with SpectrumOrange, Vysis) and Cot1 DNA (to suppress repeat sequences) was carried out on chromium coated glass chips containing microarrays of genomic clones (BAC, PAC, or P1 clones). Ratios of Green to Orange and Cy-5 to Orange, respectively, were determined for each target spot with a large field multi-color imaging system. Results with two tumor cell lines containing known gene amplifications suggest that this method establishes a correlation between gene copy number and expression level, and with further improvements may allow a much better comparison of expression levels between 2 or more cell types. Supported by the National Institute of Standards and Technology ATP Award 94-05-0021.

Program Nr: 2376 from the 1999 ASHG Annual Meeting

Sequencing analysis of deletion mutations in the giant Parkin gene. *N. Shimizu[1], N. Hattori[2], A. Shintani[1], K. Kawasaki[1], T. Kitada[1,2], S. Minoshima[1], Y. Mizuno[2], S. Asakawa[1]*. 1) Department of Molecular Biology, Keio University School of Medicine, Shinjuku-ku, Tokyo Japan 160-8582; 2) Department of Neurology, Juntendo University School of Medicine, Bunkyo-ku, Tokyo Japan 113-8421.

Recently, we identified and cloned a novel gene named Parkin which is responsible for the pathogenesis of the autosomal recessive juvenile parkinsonism (AR-JP). AR-JP patients are found to develop highly selective neuronal degeneration in the substantia nigra of brain. The Parkin gene consists of 12 exons and span over 1 Mb on the q25.2-q27 region of human chromosome 6. A variety of mutations have been found in the Parkin gene of AR-JP patients of different ethnic origins. Interestingly, the majority (75%) of Japanese AR-JP patients had large exonic deletions and mutations in the rest of patients have not been identified by the current PCR-based exon amplification assay. These unveiled mutations may include heterozygous exonic deletions, promoter mutations and others. Thus, it was desired to design appropriate PCR primers to amplify the sequence corresponding to the exact breakpoints of each deletion and the sequence of promoter region of Parkin gene. It was also necessary to design appropriate probes to detect these mutations using non-PCR methods such as FISH and Southern blotting. For this, we started sequencing the entire Parkin gene of over 1 Mb in sharing the task with The Sanger Centre. We constructed a BAC contig covering the entire Parkin gene and began sequencing the two selected regions covering exon 1 and exon 3 through exon 7. The Sanger Centre is in charge of the other two regions covering exon 2 and exon 8 through exon 12. Based on the sequences so far determined for exon 2 through exon 4, we designed 71 pairs of PCR primer and generated a detailed deletion map using DNAs from 23 Japanese AR-JP patients. We also determined the flamking sequences of the breakpoints in two patients. Moreover, we were able to design FISH probes to detect genomic DNA segments flanking each deleted exon. Molecular natures of these large deletions will be discussed.

Program Nr: 2377 from the 1999 ASHG Annual Meeting

Comparative Analysis of the Human Genome Using Primers Specific for Avian Expressed Sequence Tag Sites.

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Gene discovery and SNP identification remain major priorities of the human genetics community. In the present project, 120 primers specific for chicken embryo and turkey pituitary expressed sequence tags were used to amplify fragments in humans that were sequenced and analyzed for homology and nucleotide variants. Though seventy percent of the primers amplified products, only 15% of the amplified fragments showed significant homology to the source avian sequence. However, 90% of the human sequences showed significant homology to previously reported DNA sequences in GenBank. Five of the ESTs were mapped in the human genome using the Gene Bridge 4 radiation hybrid panel. Of the 28 SNPs discovered in the analyzed sequences, 80% were validated by allele-specific PCR and the sequencing of a second PCR product. The validated SNPs have been submitted to NCBI's dbSNP. The sequences, as well as the SNPs, provide a resource for additional comparative genome analysis of avians and humans. This information may be useful for the understanding of such processes as human development by studying the chick.

Program Nr: 2378 from the 1999 ASHG Annual Meeting

Physical/transcript map of the hereditary prostate cancer locus at Xq27.3-q28. *D.A. Stephan[1], L. Malechek[1], D. Gildea[1], J. Smith[1], M. Heiskanen[1], M.I. Quesenberry[1], J. Schleutker[1], R. Sood[1], H. Pinket[1], C.M. Robbins[1], N. Scott[1], J.D. Carpten[1], P. Meltzer[1], O. Kallioniemi[1], W.B. Isaacs[2], J.M. Trent[1]*. 1) Lab Of Cancer Genetics, NIH/NHGRI, Bethesda, MD; 2) Department of Urology, Johns Hopkins University, Baltimore, MD.

Recent identification of a hereditary prostate cancer locus at Xq27.3-q28 (HPCX) by linkage analysis in several large cohorts (Xu et al. 1998) has prompted physical mapping of the region. The maximum multipoint LOD score is at marker DXS1200 and extends several cM in either direction. Given the high phenocopy rate, we were unable to definitively localize the boundaries with trustworthy recombinants. We have therefore built a physical map of the 12 Mb region surrounding DXS1200 (DXS1192-DXS1193) in YACs, BACs and PACs, a region which extends over the entire positive LOD score. Prexisting YAC contigs were utilized as framework maps to place STSs. These STSs were used to screen BAC/PAC libraries by PCR and hybridization to identify preliminary contigs. Joins were made by BAC/PAC end-sequencing and further rounds of screening with end-STSs. Overlaps were reconfirmed by fingerprinting of all clones. ESTs and transcripts were mapped to high resolution by placement onto the BAC/PAC contig by PCR. Finally, finished sequence from the region was annotated by BLAST and exon-prediction analysis to yield further coding sequence. We have extended to full-length and characterized several of the ESTs in this interval. The analysis has shown several regions of high gene density in a mostly gene poor region. Complex genomic events such as inverted duplications have been seen and investigated by fiber-FISH. We present a sequence-ready BAC/PAC contig of 12Mb of Xq27.3-q28, a region of the genome harboring many disease-causing genes, as a resource for the identification of the HPCX gene.

Program Nr: 2379 from the 1999 ASHG Annual Meeting

Construction of a physical map of 2cen-q13 critical region containing the Primary Open Angle Glaucoma (GLC1B) locus and mutation screening of nine candidate genes. *D. Stoilova[1], A. Child[2], G. Brice[2], M. Rocchi[3], M. Sarfarazi[1]*. 1) Molecular Ophthalmic Genetics Laboratory, University of Connecticut Health Center, Farmington, CT; 2) Department of Cardiological Sciences, St. George's Hospital, London, UK; 3) Instituto Di Genetica, Universita di Bari, Bari, Italy.

Glaucoma is an ocular neuropathy affecting over sixty million individuals worldwide. Primary Open Angle Glaucoma (POAG), the most frequent form, is characterized by optic nerve damage, visual field loss and often elevated intraocular pressures. Six POAG loci have already been reported, but only mutations in the TIGR/MYOC gene are known to be associated with the glaucoma phenotype at the GLC1A locus. In order to assist the identification of causative gene for the GLC1B locus on 2cen-q13, a detailed STS-based physical map of the region has been assembled. This map consists of 38 YACs, 18 chromosome 2 specific Radiation Hybrid (RH) clones, 42 STRPs/STSs, 69 ESTs and 23 known genes. Two potential gaps in the YAC coverage have been identified in the vicinity of centromere. The first gap is between WI-9976 and WI-8267 and the second one is between GATA6GO2 and SHGC-33807. Two internal deletions were also identified for YACs 914H7 and 937D1. The physical map constructed here, localized a number of new genes and ESTs at much better resolution and made it possible to evaluate their candidacy for the GLC1B phenotype. Ten genes were mapped outside of the critical region and 17 others were mapped within the GLC1B minimum interval. Since the flanking region of GLC1B is around 9 cM, the number of potential candidate genes still remains large. A total of 9 genes from the GLC1B minimum interval, including Beta-Centractin, COX5B and ADRA2B were screened for mutations by direct sequencing but no causative mutations were identified. However, a number of polymorphisms were detected that can serve as SNPs. Screening of the 8 remaining genes is currently in progress. As more of the existing candidate genes are being excluded, identification of the causative GLC1B gene may have to rely on positional cloning. The new integrated YAC/STS and RH map provides a useful tool for this purpose. Support: EY-09947, IGA, InSite Vision, Inc.

Program Nr: 2380 from the 1999 ASHG Annual Meeting

Analysis of gene-expression profiles of single cells. *T. Suzuki[1,2,3], H. Hashida[1,2], Z. Jeong[1,2], N. Masuda[1,2], H. Murakami[2], K. Ogata[2], J. Goto[1,2], I. Kanazawa[1,2].* 1) CREST, Japan Science and Technology Corporation, Tokyo, Japan; 2) Department of Neurology, Graduate School of Medicine, Tokyo, japan; 3) Laboratory of Medical Zoology, Nagoya City Medical School, Nagoya, Japan.

In order to analyze gene-expression profiles of single cells, we have developed laser-dissection system and differential display method that can be applied to single cells. In this system, we mark target cells of freeze-dried sections on computer and laser cut out those cells automatically. For differential display of 100 cells-level RNA, increasing the cycles of PCR up to 40 cycles was revealed to be effective, while not to be effective for differential display of single-cell level RNA. For clarifying the gene-expression profiles of single cells, the "2nd round of PCR" was shown to be effective. In brief, after the 1st round of 30 cycles of cDNA amplification, nested FITC-labeled primer was used for 2nd round of differential PCR of 30 cycles. This method was successfully applied to analyses of gene expression of human and rat Purkinje cells and African Trypanosomes, protozoan parasites.

Program Nr: 2381 from the 1999 ASHG Annual Meeting

SPG4: a recombination event narrows the minimum candidate region. *I.K. Svenson[1], M.A. Nance[2], J.L. Haines[3], W.K. Scott[1], M.A. Pericak-Vance[1], D.A. Marchuk[1]*. 1) Duke University Medical Center, Durham, NC; 2) Park Nicollet Clinic, Minneapolis, MN; 3) Vanderbilt University Medical Center, Nashville, TN.

The hereditary spastic paraplegias (SPGs) are a clinically and genetically heterogeneous group of neurodegenerative disorders characterized primarily by progressive weakness and spasticity of the lower limbs. A genetic locus for the most common form of autosomal dominant SPG, SPG4, has been mapped to a 3cM interval bounded by D2S352 and D2S367 on the short arm of chromosome 2. We have identified a recombination event in a previously described family (Nance et al., Hum. Hered. 48:169-178, 1998) that narrows the SPG4 minimum candidate region (MCR) by placing its centromeric boundary at D2S2347. The markers used in the haplotype analysis in this family include seven that map between D2S352 and D2S367, with marker order determined by physical mapping to a contig of 28 YACs that span the new MCR with at least eight-fold coverage. A minimum tiling path across the new MCR consists of two overlapping megabase-sized YACs. We have mapped a total of 22 polymorphic and nonpolymorphic markers to the new MCR on the YAC contig, of which 13 are ESTs and two are associated with known genes.

Program Nr: 2382 from the 1999 ASHG Annual Meeting

Consortium to clone, map, and study human mitochondrial ribosomal protein genes. *J.E. Sylvester[1], H.-R. Graack[2], J. Liu[3], E.B. Mougey[1], B.A. Maguire[1], N. Fischel-Ghodsian[4], B. Wittmann-Liebold[5], T.W. O'Brien[3]*. 1) Nemours Children's Clinic, Jacksonville, FL; 2) Institute for Genetics, AG Kress, Free University of Berlin, Germany; 3) University of Florida, Gainesville, FL; 4) Cedars-Sinai Medical Center, Los Angeles, CA; 5) Max-Delbruck-Center for Molecular Medicine, Berlin, Germany.

Mammalian mitochondria have their own separate translation system comprised of components distinct from their cytoplasmic counterparts. Whereas mitochondrial DNA encodes tRNAs and two rRNAs, the remaining genes for the translation system are found in nuclear DNA. Upward of 100 mitochondrial ribosomal proteins (MRPs) are imported into the mitochondria, assembled into ribosomes which are responsible for translating the 13 mRNAs for oxidative phosphorylation proteins. 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. Therefore, the first step is to identify, clone, and map the gene for each human MRP. Our approach is to use N-terminal and internal amino acid sequence data obtained from purified bovine or rat MRPs (Goldschmidt-Reisin, et al., J.Biol.Chem. 273: 34828, 1998) to search EST databases. A representative I.M.A.G.E. clone (ATTC) is purchased and used to screen a human lambda genomic library. Chromosome map positions are ascertained by using *in silico* methods to search Genbank and GeneMap through NCBI, by *in situ* hybridization (FISH) with genomic sequences, and/or by radiation hybrid mapping. At present, we have over 30 different human MRPs at various stages of characterization and have established a consortium of investigators to complete the project. We are currently investigating one MRP as a potential candidate for Russell-Silver Syndrome (RSS)(see abstract, Mougey, et al., this meeting). In addition to studying their clinical relevance, long term characterization of MRP genes should lead to important insights into mammalian evolution, coordinant regulation of nuclear and mitochondrial gene expression, and ribosome function. [Supported by NIH RO1-04092 (NFG, TWO) and Nemours Research Programs (JES, EBM, BAM)].

Program Nr: 2383 from the 1999 ASHG Annual Meeting

Fiber-FISH physical mapping in the PGL1 interval involved in hereditary paragangliomas of the head and neck region. *P.E.M. Taschner[1], B.E. Baysal[2], J.G. Dauwerse[1], A. Bosch[1], J.C. Jansen[3], A.G.L. Van der Mey[3], C.J. Cornelisse[4], B. Devlin[2], P. Devilee[1,4].* 1) Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands; 2) Department of Psychiatry, Western Psychiatric Institute and Clinic, University of Pittsburgh Medical Center, Pittsburgh, PA; 3) Department of Otorhinolaryngology,Leiden University Medical Center, Leiden, Netherlands; 4) Department of Pathology, Leiden University Medical Center, Leiden, Netherlands.

Hereditary paragangliomas (MIM 168000) of the head and neck region are benign slow-growing tumors of the parasympathetic paraganglion system. The inheritance pattern is autosomal dominant with imprinting: only paternal transmission of the mutation results in tumor development. Paragangliomas are genetically heterogeneous. Most cases are linked to the PGL1 locus on chromosome 11q23. While the PGL1 gene was previously localized to a 7 cM interval between D11S1647 and D11S622, recent advances by our group refined the critical region to 1.5 Mb, between D11S1986 and D11S1347 (Hum. Genet. 104: 219 (1999)). Baysal et al (unpublished) built a YAC-BAC-PAC map of this region. More than half of the region is covered in BACs and PACs; there is complete coverage over the telomeric part, which preliminary evidence suggests contains PGL1. The continuity of the PGL1 contig could be confirmed by pulsed field gel electrophoresis of digested human DNA, followed by Southern blot hybridization using genomic clones as probes. Here, we decided to use the more efficient fiber-FISH method to refine the order and overlap of clones from the telomeric region. No chimeric clones were found by metaphase FISH. Fiber-FISH with probes in different colors confirmed the order determined by STS-based methods. Overlaps for each clone with at least one other were observed, except for the most telomeric clone. STS-PCR and Southern blotting suggested that this clone was separated from the next by a gap of 50-100 kb. No gap between these clones was seen on DNA fibers, suggesting that the gap is 5 kb at most. Fiber-FISH experiments using patient cell lines have yet to reveal any rearrangements as the cause of paraganglioma.

Program Nr: 2384 from the 1999 ASHG Annual Meeting

MICROMAX: A complete system for rapid high sensitivity gene expression analysis. *W.W. Tian, B.A. Brown, J. Killian.* Nen Life Science Products Inc, Boston, MA.

The rapid study of the entire panorama of mRNA expression in a wide range types of samples, from cell culture to clinical micro-metastasis has been enabled by the development of differential gene expression on cDNA microarrays and related chip technology utilizing oligonucleotides. However, full acceptance of this technology has been hindered by a number of factors; unavailability of genes and spotting capability, lack of adequate sensitivity, poor reproducibility, complex lengthy protocols and cost. NEN, in collaboration with AlphaGene, have introduced MICROMAX; a complete cDNA microarray system for high through put, high sensitivity differential gene expression, available to all researchers. AlphaGene provides the capability of producing high quality full length genes, with state of the art bioinformatics characterization as well as a fully optimized and reproducible cDNA microarray manufacture. NEN brings extensive assay development capability and the patented Tyramide Signal Amplification (TSA) to provide unparalleled sensitivity and convenience to cDNA microarrays. Utilizing the first product, an array of 2400 known human genes, collaborators have been able to reduce the amount of starting total RNA from the typical 100 ug to as little as 1-4 ug while gaining the capability of detecting a single copy mRNA from samples as small of 105 cells without the need to amplify the mRNA. The linear range of the response in MICROMAX is over 2 orders of magnitude and for genes that have been reported in the literature, the results with human tissue samples and cell lines agree with published Northern analysis. The reproducibility is such that significant changes in expression levels are as little as two fold. The reading of a MICROMAX slide is by standard microarray readers as it is developed with the traditional cyanine 3 and cyanine 5 dyes, though a slide reading service is available for those who do not have access to that instrumentation. With MICROMAX, any researcher can take advantage of the potential of microarray analysis of differential gene expression with a minimal investment in capital instrumentation or in the time required for optimization.

Program Nr: 2385 from the 1999 ASHG Annual Meeting

Cosmid-contig construction from the bipolar disease region at 18q21.33-q22. *F. Tissir, S. Villafurte, D. Goossens, C. Van Broeckhoven, J. Del-Favero.* Flanders Interuniversity Institute for Biotechnology (VIB),University of Antwerp, Department of Biochemistry, Psychiatric Genetics group, Antwerp, Belgium.

Linkage of bipolar disorder (BP) to chromosome 18 has been reported by several independent studies. To replicate these findings, linkage analysis with chromosome 18 markers in 10 Belgian families with a BP proband was performed. In one family, MAD 31, we found suggestive linkage to chromosome 18q21.33-q23. Multipoint linkage analysis gave the highest lod scores in a 12 cM interval between D18S51 and D18S61. Additional markers within this interval were typed in MAD 31 and the candidate region was refined to 8.9 cM. A YAC-contig map was constructed for this region with an estimated physical length of 5 Mb. Three YACs identifying the minimal tiling path were characterized by metaphase FISH analysis and shown to be chromosome 18q21.33-18q23 specific. These YACs were subcloned in the multiple exon trap cosmid vector wich is used for region specific isolation of novel expressed sequences. The cosmid libraries with a 5-8 times coverage were screened for all available markers by radioactive oligonucleotide hybridization and subsequently confirmed by PCR. To construct a cosmid-contig, DNA prepared from 96 cosmid clones is simultaneously digested and fluorescently labelled in one tube reaction. The digestion products are analysed using the fingerprinted contigs (FPC) software.

Program Nr: 2386 from the 1999 ASHG Annual Meeting

Parallel expression analyses of DNA repair genes in adult mouse brain and testis using cDNA microarrays. *L.M Tomascik Cheeseman[1,2], R. Raja[1], X. Lowe[1], J. Nath[2], A.J. Wyrobek[1]*. 1) Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, CA; 2) Genetics and Developmental Biology Program, West Virginia University, Morgantown, WV.

The objective of this research was to investigate functional variations in the expression of DNA repair genes using cDNA expression microarrays. These arrays were comprised of expressed sequence tags (ESTs) representing genes involved in double strand break repair, nucleotide excision repair, base excision repair, mismatch repair, direct reversal of damage, damage response, cell cycle regulation and apoptosis. Pooled mRNA was extracted from the adult mouse testis and adult mouse brain, reverse transcribed, fluorescently labeled with Alexa-red and Alexa-green and hybridized onto the arrays. Several genes showed differential expression (ranging from 2 to 11 fold) between the two tissues. For selected genes, northern blot analyses were performed to confirm differential gene expression between the testis and the brain. These findings have contributed to our understanding of the multiple functions of DNA repair genes in various tissues and may also have implications for cancer and aging. [This work was conducted under the auspices of the U.S. DOE by the Lawrence Livermore National Lab. under contract W-7405-ENG-48 with support from the National Institutes of Health grant ES09117-02, the University of California and West Virginia University.].

Program Nr: 2387 from the 1999 ASHG Annual Meeting

Chromosomal localization by PRINS of single copy genes SRY and SOX3. *Y. Tunca, J.S.. Kadandale, A.T. Tharapel.* Clinical and Molecular Cytogenetics Laboratory, Department of Pediatrics, University of Tennessee, Memphis, TN.

Single copy gene sequences are frequently identified with the polymerase chain reaction (PCR). Primed in situ labeling (PRINS), a specific and sensitive technique, can be adapted to the localization of single copy genes and DNA segments. Using PRINS, we have localized the 2.1kb SRY and 2.4kb SOX3 genes at bands Yp11.31p11.32 (ISCN 1995) and Xq26-q27, respectively. The SRY and SOX3 genes were selected because of their relatively small size, single exon nature and lack of commercial probes for conventional FISH. We used locus-specific oligonucleotide primers; annealing and extension were performed on day-old chromosome preparations on microscope slides, in the presence of dATP, dCTP, dGTP, dTTP and digoxigenin-11-dUTP, Tris-HCl, KCl, MgCl2, BSA and Taq DNA polymerase . The SRY signals were detected in about 95% of metaphase spreads and in about 90% of nuclei. None were detected in females. SOX3 showed one signal on the X chromosome in males and two signals on the X chromosomes in females. The PRINS technique used in this study is faster, more specific and more sensitive than conventional FISH and can be employed in cytogenetics and molecular genetics laboratories. With appropriate oligonucleotide primers, PRINS can be used for the analysis of gene deletions known in clinical entities, and for rapid localization of DNA segments.

Program Nr: 2388 from the 1999 ASHG Annual Meeting

The low-copy repeats flanking the WBS deletion and other regions in chromosome 7 contain truncated copies of *STAG3*, a gene involved in meiotic chromosome pairing. *M.C. Valero[1], N. Pezzi[2], I. Prieto[2], J.L. Barbero[2], L.A. Pérez Jurado[1]*. 1) Serv. Genética, Hosp. Univ. La Paz, Madrid, Spain; 2) Dept. Inmunología y Oncología, CNB, Madrid, Spain.

Williams-Beuren syndrome (WBS) is a contiguous gene syndrome caused by a microdeletion in chromosome band 7q11.23. It occurs with a estimated frequency of 1 in 20000 live births. Most cases are sporadic indicating a deletion rate ~ 0.5×10^{-5}. A duplicated genomic region flanks the WBS deletion interval and contains at least two genes at the telomeric block (*GTF2I* and *NCF1*), and one or more copies of their pseudogenes at the centromeric block. In addition, low-copy repeats containing genes related to the mismatch repair gene *PMS2* map to those duplications and to other chromosome 7 locations. We have identified truncated copies of another gene that are also part of the low-copy repeats. The ancestral gene, *STAG3*, encodes a meiotic-specific protein with a evolutionarily conserved domain, the stromalin conservative domain (SCD), that is involved in chromosome pairing and maintenance of the synaptonemal complex. The *STAG3* locus consists of 34 exons encompassing > 40 kb and maps to chromosome 7q22. *STAG3*-like genes consist of different sets of *STAG3* exons displaying 90-95% sequence identity and are ubiquitously expressed in all tissues tested. The *STAG3*-like loci are located in the vicinity of the 7q11.23 WBS deletion breakpoints, containing the *D7S489A* and *C* loci, as well as in 7q22. Some *STAG3L* cDNAs have short potential ORFs that might encode 134 aa proteins with the SCD. The *STAG3*-like genes are transcribed from the opposite strand of PMS2-like genes, using a common promoter region embedded in a CpG island. The mouse *Stag3* locus is single copy and maps to chromosome 5, in a region of conserved synteny with human 7q22. The presence of large duplicated segments on human chromosome 7q that are absent in mouse suggest a mechanism of chromosome evolution through subsequent duplications and provides a mechanism for unequal homologous recombination that may mediate the rearrangements responsible for WBS deletions and other cytogenetic abnormalities.

Program Nr: 2389 from the 1999 ASHG Annual Meeting

Resource Center / Primary Database of the German Human Genome Project. *A. Vente, P. Kioschis, B. Korn, C. Maurer, G. Zehetner, A. Poustka, H. Lehrach.* Resource Center German Human Genome Project, Berlin & Heidelberg, Germany.

Since 1995 the the Resource Center / Primary Database (RZPD) serves as a central infrastructural unit of the German Human Genome Project (DHGP), which was launched by the Federal Ministry of Education and Science (BMBF) and the Deutsche Forschungsgemeinschaft (DFG) for the systematical identification and characterization of the structure, function and regulation of human genes, in particular those of medical relevance. Currently approximately 50 research projects within the various fields of gene technology are supported. Established at the Max Planck Institute for Molecular Genetics, Berlin, and the German Cancer Research Center, Heidelberg, the RZPD serves as a central source of standardized reference materials for all the different DHGP projects as well as academical and industrial research groups from all over the world. Amongst the main tasks of the RZPD are the construction and collection of genomic and cDNA clone libraries from human or various model organisms and the distribution of single clones as well as high density colony filters or clone pools for the screening of these libraries. The RZPD offers a unique resource for the analysis of the human genome: Filters and DNA-pools for more than 50 human cDNA libraries from different organs and developmental stages and high density protein arrays for the screening of proteins encoded by cDNA expression libraries are available at the RZPD. The RZPD also offers a custom screening service and a service for the custom rearraying of gridded libraries. To support the various high throughput methods for the expression profiling of genes, non-redundant EST sets of different model organisms were either imported or set up at the Resource Center. The RZPD has access to clone sets for human (35,000 ESTs), mouse (25,000 ESTs), rat (25,000 ESTs), zebrafish (24,000 ESTs) and arabidopsis (12,000 ESTs). DNA-filters of these EST-sets can be requested from the Resource Center. Experimental data generated by the users of the different materials are collected by the Primary Database. These data are publicly available after an optional period of confidentiality. World Wide Web: http://www.rzpd.de email: info@rzpd.de.

Program Nr: 2390 from the 1999 ASHG Annual Meeting

Physical map of the RP11 locus for autosomal dominant retinitis pigmentosa and investigation of a candidate gene. *E.N. Vithana[1], M. al-Maghtheh[1], N. Kenmochi[2], S. Higa[2], S.S Bhattacharya[1]*. 1) Dept of Molecular Genetics, Inst of Ophthalmology, University College London, London, England; 2) Dept of Biochemistry, School of Medicine, University of the Ryukyus, Japan.

Autosomal dominant Retinitis Pigmentosa (adRP) is an inherited progressive retinal degeneration characterised by night blindness and constricted visual fields. adRP is genetically heterogeneous, whilst mutations have only been found in four genes to date genetic linkage studies have implicated at least six further loci. adRP locus on chromosome 19q13.4 (RP11) was first reported in a single large British pedigree in our laboratory. We have subsequently identified four other British families, plus a Russian and a Pakistani family linked to the chromosome 19q locus. These data together with the publication of a Japanese and three other American families also linked to 19q implicate RP11 as a major adRP locus. All linked families exhibit incomplete penetrance, individuals with the disease haplotype were either severely affected or asymptomatic. Recombination events in our RP11 families have localised the disease gene to a 3cM genetic interval between the markers D19S927(proximal) and D19S781.2(distal). In the effort to find the disease gene we have established a DNA contig spanning the RP11 interval composed of YAC, BAC, PAC and cosmid clones. Altogether the RP11 contig orders 27 STSs from a wide range of sources, including 14 microsatellite markers, 6 end clones, 3 expressed sequence tags (ESTs), 7 STS, and three known genes within a 2Mb genomic interval. The gene for ribosomal protein S9 (*RPS9*) located within the disease interval show expression in the retina but has yet undefined function. We characterised *RPS9*, which consists of 5 exons, and screened it for mutations in all RP11 linked families by direct sequencing. Apart from several polymorphisms in the non coding regions no disease causing mutations were identified thus leading to the exclusion of *RPS9*. Currently the physical map is being used to map ESTs to be evaluated as candidates for RP11.

Program Nr: 2391 from the 1999 ASHG Annual Meeting

Discovery of single nucleotide polymorphisms (SNPs) in biochemical pathway genes as risk factors for cancer.

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We are discovering novel single nucleotide polymorphisms (SNPs) in genes relevant to cancer. Our goal is to determine the potential relationship between these variants and cancers of the liver, breast, ovary and prostate. We are taking a "pathway-directed" approach, targeting genes for SNP characterization that are part of well described biochemical pathways that determine how an individual responds to endogenous and exogenous exposures.

The SNPs are found by data mining of publicly available DNA sequences. Trace files are retrieved from the Wash. U. Genome Sequencing Center based on significant identity with a "seed" DNA sequence for each gene of interest. A suite of software tools, previously described as the "SNP pipeline" (Nature Genetics, 21, 323-325, 1999) is used to detect single nucleotide variants in the resulting sequence assemblies. Primers are designed to flank the predicted SNP, which are validated by restriction enzyme digestion or sequencing of individuals in CEPH families. Variants are designated as "confirmed SNPs" following observation of Mendelian transmission in CEPH families and placement on reference genetic maps.

We have predicted and confirmed single nucleotide polymorphisms in several genes implicated in liver, breast, ovarian and prostate cancer. These include previously undescribed variants in the catechol-O-methyltransferase (COMT) gene resulting in silent mutations (His62His, Leu136Leu), and variants in the 3' UTR of GSTA4 and GSTT1. In addition to the identification of novel SNPs, we have also observed several previously described polymorphisms, including functional mutations within COMT (Val158Met), GST pi (Ala114Val) and GST zeta (Gly42Arg, Thr82Met) and variants within the 3' UTR of CYP19 and GSTT2. Detection of known single nucleotide polymorphisms in these genes supports the validity of this approach in the discovery of new variants.

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Program Nr: 2392 from the 1999 ASHG Annual Meeting

Transcript map of a genetic locus at 11q23 linked to hypoalphalipoproteinemia. *S. Wagner[1], W. Ding[1], H. Wang[1], H. Goldfine[1], T. Rigley[1], R. Kehrer[1], M. Frodsham[1], M. Hess[1], J. Malandro[1], K. Eddington[1], B. Miao[1], M. Chen[1], K. Harshman[1], C. Schumacher[2], P. Chen[2], N. Lyons[2], D. Duricka[2], B.R. Bowen[2], M. Skolnick[1], D. Ballinger[1]*. 1) Myriad Genetics, Inc., Salt Lake City, UT; 2) Novartis Institute for Biomedical Research, Summit, NJ.

Epidemiological evidence shows a strong inverse correlation between the level of plasma high-density lipoprotein (HDL) and the incidence of heart disease. It has been suggested that HDL has a protective effect against cardiovascular disease, and hypoalphalipoproteinemia is considered a potent predictor of coronary heart disease. Several genetic deficiencies have been linked to extreme hypoalphalipoproteinemia, however no variants that account for the more common forms of low HDL have been described. Genetic analysis of Utah pedigrees with familial coronary heart disease located a susceptibility locus for hypoalphalipoproteinemia in a 10cM region on 11q23. Initial genetic mapping defined a region between D11S924 and D11S934 which was further refined by recombinants to a 2Mb minimal interval between D11S1353 and D11S933. Physical mapping of the minimal region assembled a minimal tiling path of 16 BACs and PACs. Transcription units were identified by a combination of large scale automated sequencing and a set of complementary methods for the isolation of transcribed sequences. We describe here an integrated physical and transcript map of the minimal recombination interval. Fourteen genes and one pseudogene were identified and characterized. Five genes were identical to mRNAs described in Genbank (SP17, neurogranin, BCSC-1, ZNF202, CTH). One transcript shows strong similarity to the mouse gene for sialic-acid acetyl-esterase and most likely represents the human homolog. One gene shares strong homologies with the b subunits of known sodium channel proteins, but appears to be a novel gene. Seven transcripts are novel genes of unknown function. A cluster of putative olfactory receptor genes is located in the center of the region including a number of pseudogenes. Biochemical evidence suggests that ZNF202 is a potent regulator of lipid metabolism and a strong candidate for a hypoalphalipoproteinemia predisposition gene.

Program Nr: 2393 from the 1999 ASHG Annual Meeting

Physical and transcript mapping of the Blau syndrome susceptibility locus. *X. Wang[1], G. Tromp[1], H. Kuivaniemi[1], L. Molina[2], L. Serrano de la Peña[2], C.J. Williams[2].* 1) Ctr. Molec. Med. and Genet., Wayne State University, Detroit, MI; 2) Div. Rheumatology, Thomas Jefferson University, Philadelphia, PA.

Blau syndrome is a multi-system inflammatory disease that is characterized by the development of granulomas in the joints, eyes, and/or skin. The disease is inherited in an autosomal dominant manner and we have used genetic linkage analysis to map the disease susceptibility locus to the long arm of chromosome 16. The most likely disease interval resides in an 8 cM region demarcated by the microsatellite markers D16S411 and D16S415, and coincides with a Crohn's disease locus linked to chromosome 16q. Although chromosome 16 is one of the most well-characterized of the human chromosomes, there appears to be some inconsistency with regard to the order of polymorphic markers in the candidate interval; this inconsistency has hampered our efforts at refined linkage mapping. To alleviate these discrepancies, we have undertaken refined mapping of the markers in and around the susceptibility interval using high and medium resolution radiation hybrid panels (TNG4 and G3) and by concurrently constructing a low resolution YAC contig and a high resolution BAC contig of the interval. We have also used these resources to refine the chromosomal location of several potential candidate genes for Blau syndrome: we have determined that the genes for the matrix metalloproteinases MMP2 and MMP15 reside outside of the susceptibility interval, as does the gene for carboxyesterase 1 (CES1). We will continue to utilize the physical mapping resources in the Blau susceptibility interval to fine map additional potential candidate genes, as well as ESTs that have been provisionally assigned to the locus. (Supported by a Biomedical Science Grant from the Arthritis Foundation).

Program Nr: 2394 from the 1999 ASHG Annual Meeting

An 8kb deletion/insertion polymorphism in the Van der Woude syndrome critical region at 1q32-q41. *Y. Watanabe[1], B.C. Bjork[1], P.-W Chiang[2], S.G. Gregory[3], D.M. Kurnit[2], J.C. Murray[1], B.C. Schutte[1].* 1) Dept. of Pediatrics, University of Iowa, Iowa City, IA; 2) Dept. of Pediatrics, University of Michigan, Ann Arbor, MI; 3) Sanger Centre, Hinxton, Cambridgeshire, UK.

VWS is an autosomal dominant disorder whose cardinal features are cleft lip and/or palate and lip pits. The VWS gene was localized to a 1.6cM region of 1q32-q41 flanked by markers D1S491 and D1S205. A contig of BAC and PAC clones was constructed across the VWS critical region (B. Bjork et al., submitted). Eleven overlapping BAC and PAC clones, spanning approximately 1.1 Mb, were selected for high-throughput genomic (HTG) sequence analysis by the Sanger Centre (UK). From sequence analysis the BAC clone 321i20 and the PAC clone 782d21 overlap, however the clone 321i20 was missing a 7922 bp region. Imbedded in this 7922 bp region was a short tandem repeat (STR). To verify that this nearly 8kb deletion in the clone 321i20 was not a cloning artifact, we genotyped unaffected control samples using two sets of PCR primers, 186/7 that flank the STR and 583/4 that flank the putative 8 kb deleted sequence. The genotype data with 186/7 showed that the STR was polymorphic. The genotype data with 583/4 showed that the 8 kb deletion mutation was present in 54% of the chromosomes tested. The presence of the 8 kb deletion in the control samples was confirmed by QPCR. These results demonstrate the presence of an STRP within a common 8 kb deletion/insertion at the distal end of the VWS critical region. In addition, the deletion breakpoint sequences from 11 unrelated individuals were identical, suggesting that the 8 kb deletion/insertion may be an old mutation. Since this polymorphism is present in control samples and appears to be an old mutation, it is not a mutation that is etiologic for VWS. However, it is located near the distal breakpoints for two known microdeletions that are etiologic for VWS, providing additional evidence that this may be a region of genetic instability.

Program Nr: 2395 from the 1999 ASHG Annual Meeting

Physical and genetic mapping of the CT locus on dog 10q26. *C. Wijmenga[1], B. van de Sluis[1], J. Rothuizen[2], B.A. van Oost[2], P.L. Pearson[1].* 1) Dept Medical Genetics, University Medical Center, Utrecht, Netherlands; 2) Dept. Veterinary Sciences, Utrecht University, Utrecht, the Netherlands.

Copper toxicosis (CT) in Bedlington terriers is an autosomal recessive disorder characterised by accumulation of copper in the liver. This terrier might be an interesting animal model for human diseases associated with hepatic copper overload, such as idiopathic copper toxicosis (ICT). The anonymous microsatellite marker C04107, which is closely linked to CT in Bedlington terriers, was isolated in a canine BAC clone and mapped to dog chromosome10q26 by FISH. Sample sequencing of the C04107 BAC revealed a subclone with high homology to one exon of the human MURR1 gene, which was used to show conservation of synteny with human 2p13-p16. Genes and ESTs known to map to human 2p13-16 are currently mapped onto the dog genome and are being used to isolate BAC clones for the construction of a physical map. Polymorphic markers from the 10q26 region have been used to locate the CT locus between two markers that are 30 cM apart. Currently, new polymorphic markers are being developed to construct a high resolution genetic map.

Program Nr: 2396 from the 1999 ASHG Annual Meeting

Single Nucleotide Polymorphism (SNP) Mapping of a Type 2 Diabetes-linked Region on Human Chromosome 1q21-q23. *J.K. Wolford, R.L. Hanson, C. Bogardus, P. Permana, M. Prochazka.* NIDDK/PECRB, NIH, Phoenix, AZ.

Type 2 diabetes mellitus is a complex heritable disease. A genetic basis for the disease is especially evident in the Pima Indians of Arizona, who have the world's highest reported prevalence of this disease. We have previously demonstrated evidence for linkage of diabetes in the Pimas in a 30 cM region on 1q21-q23 with a maximum multipoint LOD score (2.5) at *D1S1677*. This region has also been linked to type 2 diabetes in a Caucasian population (*Diabetes* 48: 1175-1182, 1999). To narrow the area of linkage and identify potential candidate genes, we are evaluating densely spaced SNP markers by association analysis in 117 Pima sib-pairs discordant for diabetes and in 100 unrelated Pimas, including 50 affected and 50 unaffected subjects. We are utilizing a contig of 65 overlapping YACs that encompasses the region of linkage and spans approximately 34 cM between markers *D1S442 and D1S452*. Using a combination of automated sequencing, DHPLC, PCR-RFLP, and AD-PCR, we have analyzed 36 SNPs. These SNPs include 13 previously reported markers and 23 novel, gene-specific markers (in *ATP1A2, ATP1B1, CRABP2, FCER1A, FCGR2A, FLG, GBA, LMX1, MCL, PEA15, RXRG, SPRR1B* and *USF1*) which were identified as polymorphic in Pimas. Four SNPs in the *GIRK3* gene are associated with diabetes (p=0.0003 in discordant sib-pairs; p=0.003 in the affected/unaffected group). *GIRK3*, located approximately 3 cM centromeric from the linkage peak at *D1S1677*, encodes a G-protein-coupled inwardly rectifying potassium channel protein. The GIRK3 protein is known to regulate ion flow and hormone secretion in some tissues and thus represents a candidate gene for diabetes susceptibility. We are scanning the *GIRK3* gene for mutations in Pimas and constructing a BAC contig through the *GIRK3-D1S1677* region to concomitantly identify additional candidate genes and locate novel SNPs for use in haplotype analysis.

Program Nr: 2397 from the 1999 ASHG Annual Meeting

Construction of a BAC Contig for Murine Chromosome 11 (32-34 cM): The Region Homologous to the Smith-Magenis Syndrome Common Deletion. *J. Yan, C.F. Boerkoel, K. Walz, J.R. Lupski.* Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Smith-Magenis syndrome (SMS) is one of the most frequent contiguous gene deletion syndromes and is characterized by mental retardation and multiple anomalies. In humans, the common deletion region is 17p11.2, which is syntenic to the 32-34 cM region of murine chromosome 11. Several genes have been mapped to both the mouse and human regions of synteny. By using 21 probes specific for these genes and genetic markers in this region, we screened the mouse RPCI-23 BAC library and isolated 96 positive clones. After PCR verification, we constructed a BAC contig spanning the mouse syntenic region. These BACs are being mapped into a sequence ready contig. In addition, we found that this region does not have sequences homologous to the human repeated gene cluster (SMS-REP) that flanks the SMS common deletion region. These unique specific low-copy repeats are a substrate for homologous recombination resulting in unequal crossing over and consequent deletion in humans. This indicates that the occurrence of SMS-REP is a recent event in mammalian genome evolution. Our physical mapping effort will enable genomic characterization of this region and is critical for constructing a mouse model for SMS.

Program Nr: 2398 from the 1999 ASHG Annual Meeting

A practical application of sample sequencing strategy and the GeneHunting software tools in positional cloning of progressive epilepsy with mental retardation (EPMR) and laryngeal carcinoma cancer region at chromosome 8p23. *Y. Zhang[1], X. Ye[1], S. Ranta[2, 3], B. Ross[1], Y. Tao[1], M. Chien[1], A. de la Chapelle[3, 4], A. Lehesjoki[2], P. Zhang[1], T. Gilliam[1]*. 1) Columbia Genome Ctr, Columbia Univ, New York, NY; 2) Dept. of Medical Genetics, University of Helsinki,; 3) Folkhälsan Institute of Genetics, Helsinki, Finland; 4) Comprehensive Cancer Center, Ohio Sate University, Columbus, OH.

One of the challenges of positional cloning is to quickly identify genes in the defined disease region. We describe a strategy that combines sample sequencing and automated computational analysis to quickly and effectively generate a transcript map. We sought to determine the extent of sequencing coverage necessary to generate a transcription map equivalent to or better than that currently available by standard molecular cloning and hybridization methods. In order to handle the large amount of sequencing data efficiently, we developed a software package, GeneHunting, on the UNIX client for DNA sequence assembling and editing, contig assembling, automatic database searching and annotation, cDNA versus Genomic DNA alignment, exon prediction, statistical report and graphic view with a user-friendly web interface. The scaffolding for the program includes PHRED/PHRAP, CONSED, CROSSMATCH, Network BLAST and GENESCAN. In addition, we integrate these tools using scripts written with PERL and JAVA. The data is automatically processed at each night. The final reports are output in HTML format and the end users can view the results with a standard web browser. Using the data that we generated in the EPMR study, we found that 1.5 X sequencing data generated comparable, or better, transcript coverage than the traditional exon trapping and cDNA selection methods. Sample sequencing combining with our GeneHunting tools provides advantages of automation, greater cost and time saving over the traditional methods. Detailed comparison of our strategy and the traditional ones, as well as the description GeneHunting interface and functionality will be presented.

Program Nr: 2399 from the 1999 ASHG Annual Meeting

Molecular characterization of FRA7E - a common fragile site on human chromosome 7. *E. Zlotorynski[1], S.W. Scherer[2], D. Mishmar[1], J. Skaug[2], A. Rahat[1], L.-P. Tsui[2], B. Kerem[1].* 1) Genetics, Life Sciences, Jerusalem, Israel; 2) Genetics, Hospital for sick children, Toronto, Canada.

Common fragile sites are specific unstable loci characterized as constrictions, gaps or breaks on chromosomes from cells exposed to replication stress. These sites were implicated in chromosomal rearrangements (both constitutive and in cancer), gene amplification, sister chromatid exchange and integration of foreign DNA. Only three common fragile sites, FRA3B, FRA7G and FRA7H, all induced by aphidicolin, have been characterized at the molecular level. In contrast to rare fragile sites, expanded repeats had not been found in common fragile sites, and the molecular basis for their fragility remains obscure. Here we report the identification and characterization of another common fragile site, FRA7E, on the long arm of human chromosome 7. Contigs of YAC, PAC and BAC clones from 7q21 were used as probes in a FISH analysis on metaphase chromosomes expressing fragile sites. Identification of hybridization signals telomeric, centromeric or on both sides of the gaps and constrictions of FRA7E, enabled us to map and define a region of ~500 kb encompassing this fragile site. Using STS markers from that region, we were able to construct a contig of >1 Mb (with only two small gaps, tens of kb long each), out of published PAC and BAC sequences, encompassing the FRA7E region. No expanded repeat sequences were identified in the sequenced region, confirming previous results indicating that the molecular basis of aphidicolin induced common fragile sites differs from that of rare fragile sites. Analysis of ORFs and potential non-B DNA structures within the region will be discussed. Interestingly, FRA7E resides a few Mb proximally to the PGY (MDR) genes, known to be amplified in various types of cancer, and underlying their resistance to chemotherapy. The involvement of FRA7E in these amplification events is currently under investigation.

Program Nr: 2400 from the 1999 ASHG Annual Meeting

Acute, fatal presentation of ornithine transcarbamylase deficiency in a 62-year-old man. *D. Kostiner, K. Weisiger, E. Moffatt, N. Linderman, R. Lamb, G. Lopez, F. Sharp, S. Goodman, M. Tuchman, S. Packman.* Medical Genetics, U. of California, San Francisco, CA.

Ornithine transcarbamylase (OTC) deficiency is an X-linked urea cycle defect. While hemizygous males typically present with hyperammonemic coma in infancy, there are case reports of late-onset presentations with poor outcomes in males 6-58 years old. Interestingly, relatives carrying the same mutations often remain asymptomatic. Multifactorial influences clearly affect disease penetrance and expressivity. We present a patient with late-onset OTC deficiency to educate clinicians about urea cycle defects in adults and to consider environmental factors that may have impacted disease expression in this patient. A previously healthy, intelligent 62-year-old man developed mental slowing, vision changes, emesis, refractory seizures and coma over a 10-day period. History was remarkable for recent use of home-gardening fertilizers and pesticides. Evaluations for drug and alcohol use, infections, and liver disease were negative. Aggressive treatment including hemodialysis was initiated for ammonia level of 457 uM (nl 11-35). Despite therapy, ammonia peaked at 2050 uM and patient expired from cerebral edema and cerebellar herniation. Urine orotic acid was 473 MMol/Mol CR (nl 0-3). Analysis of the OTC gene showed a Pro-225-Thr change in exon 7. This mutation has been previously implicated in causing OTC deficiency. Family history revealed a sister with arrested cognitive maturity at puberty and 3 normal brothers. Mutation testing of siblings is currently underway. We conclude the following: 1) Pro-225-Thr mutation can be associated with late onset OTC deficiency. 2) Exposure to pesticides and fertilizers might have contributed to onset of symptoms in this patient. Tissue levels of these organic compounds are currently being assessed. 3) Acute, severe hyperammonemia in a previously healthy adult might be due to OTC deficiency. Physician awareness of this possibility can lead to rapid treatment and appropriate genetic counseling.

Program Nr: 2401 from the 1999 ASHG Annual Meeting

Molecular pathology of bovine b**-mannosidosis.** *S.A. Kraemer[1], J.R. Leipprandt[1], M.Z. Jones[1], K.H. Friderici[2]*. 1) Division of Human Pathology, Michigan State University, East Lansing, MI; 2) Department of Microbiology, Michigan State University, East Lansing, MI.

b-Mannosidosis, a disease with autosomal recessive inheritance, results from the deficiency of lysosomal b-mannosidase activity. This deficiency is associated with severe prenatal and neonatal neurological and skeletal deformities in the naturally-occurring Salers cattle model. Although bovine b-mannosidase RNA levels in the affected animal were comparable to those in the normal control, no residual enzyme activity or protein was detected in tissues of affected animals, suggesting a total loss of function mutation, with a post-transcriptional mechanism, may cause this disease. A nonsense mutation, G2574A, associated with the bovine disease was identified. This mutation is predicted to result in the deletion of only the C-terminal 22 amino acids of the 879 amino acid bovine b-mannosidase, a region not previously predicted to be critical in the catalytic function of the enzyme. To explore the molecular basis underlying bovine b-mannosidosis, wild-type and mutant bovine b-mannosidase were expressed in CHO-K1 cells. Wild-type transfectants had significantly elevated b-mannosidase enzyme activity in both intracellular and secreted fractions; however, no activity was detected in either fraction of the mutant transfectants. Following metabolic labeling with ^{35}S-methionine and immunoprecipitation using a polyclonal anti-bovine b-mannosidase antibody, both wild-type and mutant b-mannosidase proteins were detected in the intracellular fraction. Both intracellular proteins appeared to be subjected to similar post-translational carbohydrate modification, and had similar stability. On the other hand, unlike the wild-type recombinant protein, the mutant recombinant protein was absent in the secreted fraction. These results suggest that the mutation associated with bovine b-mannosidase does not appear to interfere with proper translation or intracellular glycosylation of the protein. However, the C-terminal 22 amino acids of b-mannosidase apparently play an unforseen role in the activity, secretion and/or extracellular stability of the enzyme.

Program Nr: 2402 from the 1999 ASHG Annual Meeting

Clinical Manifestations in Three American Adult Patients with Carbohydrate-deficient Glycoprotein Syndrome.
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CDGS is a group of multisystemic disorders resulting from defective N-linked oligosaccharide synthesis. 60-70 cases of CDGS have been diagnosed in the United States over the past 5 years. Only three American adult CDGS cases have been recognized; a 24 year old woman and her 25 year old brother, and a 30 year old man, the oldest recognized American CDGS patient. All are CDGS type 1 patients, as determined by diagnostic serum transferrin isoelectric focusing. The siblings are CDGS type 1A, the most common CDGS type, as defined by lymphoblast phosphomannomutase (PMM) deficiency; PMM assay is pending in the third case. All had unremarkable birth histories followed by failure to thrive, developmental delay and esotropia recognized in the first few months of life. Their infantile medical histories revealed only mildly elevated liver function tests which persisted during their first few years of life. Hypotonia and ataxia were pronounced throughout their development; none achieved independent standing, the third patient briefly walked with a walker. Scoliosis, kyphosis and striking truncal shortening have been progressive throughout the teenage and adult years. Because of ataxia and profound skeletal involvement, currently all are wheelchair bound. All have the previously reported peripheral neuropathy with only the oldest patient having progressive muscle wasting (Stibler, 1994). Muscle strength is good, with the siblings atypically maintaining normal muscle mass. All three patients have retinitis pigmentosa (RP) and cerebellar hypoplasia/atrophy. The men have normal adult genitalia; the woman has no breast development. Their cognitive abilities are remarkable, while severely dysarthric all three carry on conversations punctuated with good natured joking. The oldest is in an assisted living setting and describes himself as an "eclectic music lover". The clinical description of these adults cautiously support a more positive cognitive prognosis in children with CDGS type1. CDGS should be considered in any adult patient with mental retardation, ataxia, truncal shortening and RP.

Program Nr: 2403 from the 1999 ASHG Annual Meeting

Polymorphisms in the CBS gene associated with increased risk of heart disease and decreased responsiveness to homocysteine lowering by folate. *W.D. Kruger[1], L. Wang[1], A.A. Evans[1], M.R. Malinow[2], P.B. Duell[2], D.L. Hess[3], R.A. Gluckman[4], P.C. Block[4], P.H. Anderson[4], B. Upson[4].* 1) Population Sci, Fox Chase Cancer Ctr, Philadelphia, PA; 2) Department of Medicine, Oregon Health Sciences Unversity, Portland Or; 3) Reproductive Sciences, Oregon Regional Primate Research Center, Beaverton Or; 4) Providence St. Vincent Medical Center, Portland Or.

Elevated total plasma homocysteine (tHcy) is an established risk factor in the development of vascular disease. Folic acid supplements can generally lower tHcy levels but individual response is highly variable. In this case-control study we have examined various genetic polymorphisms in homocysteine metabolizing genes and their relationship to incidence of coronary artery disease (CAD), tHcy levels, and lowering of tHcy levels in response to folic acid supplementation. We have found that two polymorphisms in the cystathionine beta synthase (*CBS*) gene, 699c and 1080t, are associated with increased incidence of CAD and decreased folate responsiveness. Individuals homozygous for either 699c or 1080t are over represented in CAD patients and show decreased tHcy lowering in response to treatment by folate. The two polymorphisms in *CBS* are third codon changes and would not be predicted to affect the underlying protein. Interestingly there is strong linkage disequilibrium between these two positions, suggesting that they may also be linked to other as yet unidentified polymorphisms within the *CBS* gene. These observations suggest that specific *CBS* alleles are a risk factor for the development of vascular disease. In addition, it may be possible with genetic information to predict *apriori* an individuals response to folic acid supplementation.

Program Nr: 2404 from the 1999 ASHG Annual Meeting

Perinuclear localization of hephaestin suggest exocytic intestinal iron export. *Y. Kuo[1], C.D. Vulpe[2], G.J. Anderson[3], J.G. Gitschier[1,4].* 1) Department of Pediatrics, University of California, San Francisco, CA 94143; 2) Department of Nutritional Sciences, University of California, Berkeley, CA 94720; 3) Joint Clinical Sciences Program, Queensland Institute of Medical Research and University of Queensland, Queensland, 4029; 4) Howard Hughes Medical Institute, U.C.S.F., CA 94143.

Hephaestin encodes a transmembrane-bound ceruloplasmin homolog, which is mutant in the *sla* mouse and is highly expressed in the intestine. We suggest that hephaestin is a multi-copper ferroxidase necessary for iron egress from intestinal enterocytes into the circulation. Hephaestin was localized in the mouse small intestine using affinity-purified hephaestin antiserum. The antiserum was raised in rabbits to a peptide corresponding to amino acids in the predicted cytoplasmic domain at the C-terminus. By RNA *in situ* hybridization, hephaestin is highly expressed throughout the intestinal villi but absent in the crypt cells. However, by immunostaining hephaestin is within the enterocytes of the intestinal villi in a perinuclear location. When the antiserum was preabsorbed with the peptide used for immunization, there was no detectable staining indicating the staining specificity for hephaestin protein. Perinuclear localization was confirmed by double staining with the nuclear marker propidium iodide. Double staining with anti-Na^+K^+- ATPase antibody, a marker for the plasma membrane, clearly shows that hephaestin does not localize to the plasma membrane. We conclude that in iron replete adult animals at steady state, hephaestin resides in a perinuclear compartment rather than directly on the basolateral cell surface of the enterocytes. Other proteins involved in intestinal metal export , such as ATP7A, the copper transporting ATPase defective in Menkes disease and the mottled mouse, also localizes to an intracellular compartment (trans-Golgi) within the cell. The intracellular localization of hephaestin suggests that iron export from the intestine proceeds via transport into an intracellular compartment followed by exocytosis of vesicle-bound iron. Hephaestin localization in *sla* and mottled mutant mice will also be discussed.

Program Nr: 2405 from the 1999 ASHG Annual Meeting

The arsenite-stimulated ATPase (hASNA-I) interacts with metallothionein-II and modifies the human cellular response to arsenite. *B. Kurdi-Haidar, L. Fink, D.K. Hom, D. Heath.* UCSD Cancer Center, Univ California San Diego, La Jolla, CA.

The physiologic role of metallothionein (MT), as a metal binding protein affecting zinc and copper cellular homeostasis, in human genetic disorders of Cu metabolism has been described. The human arsenite-stimulated ATPase (hASNA-I) was recently identified as a human paralogue of the ATPase component of the arsenite efflux system in E. coli. Using the hASNA-I cDNA as bait in a yeast two-hybrid system, metallothionein II (MT-II) was identified as a cellular protein interacting with hASNA-I. Three lines of evidence verified that MT-II is a true partner of hASNA-I. First, hASNA-I can be covalently cross-linked to purified MT-II. Second, GST-hASNA-1 fusion protein selectively captured both purified and cellular hMT-II in a glutathione-dependent manner. Third, affinity mass spectrometry based on surface enhanced laser desorption/ionization showed binding of purified MT-II to hASNA-I. Glutathione markedly diminished the binding of MT-II to hASNA-I. The physiological consequence of the interaction between the two proteins on the sensitivity to arsenite was investigated by over-expression of either hASNA-I or hMT-II alone or together in human squamous head and neck carcinoma UMSCC10b cells. No change in arsenite sensitivity was observed when the two proteins were expressed separately, but over-expression of both together resulted in an increase in arsenite sensitivity by a factor of 1.9-fold (p=0.02). Thus, hASNA-I appears to be a modulator of the sensitivity of human cells to arsenite in the presence of MT-II. Studies of the effect of the interaction between hASNA-I and MT-II on the cellular sensitivity to Cu are underway. The current findings raise the possibilities that: 1) hASNA-I might influence the genetic susceptibility of individuals to arsenite carcinogenesis; and, 2) the physiological role of MT in disorders of Cu metabolism could also be mediated through its interaction with other cellular partners such as hASNA-I. *E. colihASNA-IhASNA-IhMT-II*.

Program Nr: 2406 from the 1999 ASHG Annual Meeting

Nonketotic hyperglycinemia: Mutation spectra of the GLDC and AMT genes in Finnish and non-Finnish populations. *S. Kure[1], M. Takayanagi[1], Y. Kurihara[1], J. Leisti[2], D. Zalai[3], G. Chuck[4], K. Tada[5], Y. Matsubara[1], K. Narisawa[1]*. 1) Department of Medical Genetics, Tohoku University School of Medicine, Sendai, Japan; 2) Department of Clinical Genetics, Oulu University Central Hospital, Oulu, Finland; 3) Department of Pediatrics, Semmelweis University School of Medicine, Budapest, Hungary; 4) Division of Pediatric Neurology, Children's Hospital Medical Center, Cincinnati, Ohio; 5) NTT Tohoku Hospital, Sendai, Japan.

Nonketotic hyperglycinemia (NKH) is an inborn error of metabolism characterized by accumulation of a large amount of glycine in body fluid. Incidence of NKH is estimated as 1 in 250,000 births in many countries while it is unusually high in northern Finland (1 in 6,000 births). NKH is caused by deficiency of the glycine cleavage system (GCS), which consists of four individual components referred to as P, T, H, and L-proteins. Three and six mutations have been reported in the genes encoding P-protein (GLDC) and the T-protein (AMT), respectively. To elucidate the mutation spectra of NKH in Finnish and non-Finnish population we screened 27 Finnish and 33 non-Finnish patients for mutations in the GLDC and AMT genes by direct sequencing analysis of each exon. In Finnish patients we identified two prevalent mutations in the GLDC gene, S564I and G761R. The two mutations accounted for 42 of 54 Finnish mutant alleles (78 percent) and no mutation was found in the AMT gene. In non-Finnish patients, 20 and 8 patients were identified to have mutations in the GLDC and AMT genes, respectively, and no mutation was detected in 5 patients. The mutations were heterogeneous: there were 24 and 12 mutations in the GLDC and AMT genes. There results suggest a sharp contrast of genetic background of NKH in Finnish and non-Finnish populations.

Program Nr: 2407 from the 1999 ASHG Annual Meeting

The N370S/N370S glucocerebrosidase genotype is associated with low chitotriosidase activity. *L.M.W.G. Lacerda[1], O.M.O. Amaral[1], E.M. Pinto[1], E. Silva[1], P. Oliveira[2], M.C.P. Sa Miranda[1].* 1) Genetic Neurobiology, IBMC-University of Porto, Porto, Portugal; 2) Department of Production and Systems, University of Minho, Braga, Portugal.

Gaucher disease (GD), the most prevalent lysosomal storage disease, is an autosomal recessive disorder of glycosphingolipid metabolism. It is characterised by accumulation of glucosylceramide in macrophages (Gaucher cells) due to the glucocerebrosidase deficient activity. Chitotriosidase is a human chitinase produced by these cells in tissues and organs and may thus reflect the severity of the disease. Although the glucocerebrosidase (GBA) gene mutations have been extensively characterised, no correlation can be easily established between GBA genotype and the clinical expression of the disease. In this work we attempted to correlate the clinical severity, reflected by chitotriosidase activity, with GBA genotype. Patients were also screened for the frequent 24bp chitotriosidase duplication. Sixty-three Portuguese type 1 GD patients were studied and the mean chitotriosidase activity of the 38 GD patients not carrying the chitotriosidase gene mutation was 15282/-16367, ranging from 1265-68394. From these, a group of 7 patients who were homozygotes for the N370S GBA gene mutation (mean chitotriosidase activity 4223/-1895) presented significantly lower chitotriosidase activity than that presented by the 31 patients with other GBA genotypes (mean chitotriosidase activity 17780+/-17166). This finding substantiates further our previous prediction of mild clinical presentation in this genotype group. Twenty-five GD patients are under enzyme supplementation therapy and the biochemical follow-up was done by comparing changes of plasma chitotriosidase activity. The importance for the establishment of the chitotriosidase deficiency carrier status was manifold: to evaluate the starting dose/regimen, on the basis the initial chitotriosidase activity (which reflects the overall Gaucher cell burden in the body); to establish the lowest effective dose, by making use the of maximal reduction of chitotriosidase activity as a marker of the correction and/or prevention of ongoing formation of Gaucher cells.

Program Nr: 2408 from the 1999 ASHG Annual Meeting

Tissue-Specific Regulation of Human Galactose-1-Phosphate Uridyltransferase (GALT). *K. Lai, C.A. Presley, S.D. Langley, L.J. Elsas.* Dept Ped, Div Medical Genetics, Emory Univ, Atlanta, GA.

Classic Galactosemia in humans is an inherited metabolic disorder caused by deficiency of GALT. While a galactose-restricted diet can prevent a lethal hepatotoxic syndrome, long-term complications such as verbal dyspraxia, ataxia, mental & growth retardation, and premature ovarian failure may occur. We have defined differences in organ expression of GALT enzyme activity (Lai *et al.*, 1996, *J. Pediatrics* v128: 89-95). By Northern blot analysis, GALT mRNA abundance varies. We found abundant GALT message in liver, ovary, brain, heart, while little or no message is in pancreas, skeletal muscle. To understand the molecular mechanisms for tissue-specific expression of the human GALT gene, we cloned and sequenced 4 kb of DNA 5' to the translation start site. Using the TRANSFAC v3.2 program, we found numerous putative binding sites for tissue-specific and developmentally-regulated transcriptional factors such as HNF-3, HNF4, HNF-5, IRF-1. No TATA box was found. Primer extension analysis using RNA harvested from normal human fibroblasts, and liver tissues, as well as three tissue-specific cell lines HEK293 (embryonic kidney), NIH-OVCAR-3 (ovary) and HepG2 (liver) indicated numerous transcription initiation sites. The strongest mRNA start signal was at a GC-rich region located at -80 nucleotide (nt) relative to the ATG start site. Functional analyses of the GALT gene promoter using a luciferase reporter gene system and the tissue-specific cell lines showed predominant promoter elements within the 166 nucleotides (-1nt to -166nt) 5' to the ATG start. A fibroblast-specific repressor binding site was between -166nt and -366nt. Using a construct containing -1nt to -698nt, we found 100 fold higher reporter activity in the embryonic kidney cell lines compared to liver, ovary and fibroblasts. We conclude that the human GALT gene expression is regulated in a tissue-specific and developmental manner.

Program Nr: 2409 from the 1999 ASHG Annual Meeting

A rapid and direct detection for the CYP21P/CYP21 chimeric gene in steroid 21-hydroxylase deficiency: an analysis for PCR dropout. *H.H. Lee[1], J.G. Chang[1], C.H. Tsai[1], B.c. Chung[2]*. 1) Deptment of Medical Research, China Medical college Hospital, Taichung, Taiwan, R.O.C; 2) Institute of molecular Biology, Academia Sinica, Nankang, Taipei, Taiwan, R.O.C.

We have developed a rapid and direct method to detect chimeric CYP21P/CYP21 gene, which resulting from gene crossover, in congenital adrenal hyperplasia (CAH) using a PCR-based assay. The strategy was to use primers containing a 3' specific primer for the CYP21 gene and two different 5' primers for both the normal CYP21 and the CYP21P of the chimeric CYP21P/CYP21 genes in the PCR amplification. For detecting gene crossover region, a secondary PCR was performed using another set of primers which included one 5' primer specific for the chimeric gene and the other for the normal CYP21 gene. The size of the PCR products was able to differentiate chimeric gene from the normal gene. By adding a CYP21/CYP21P specific primer for the 8 base-deleted region (codon 111-113) in intron 2, we were able to amplify and detect both the chimeric homozygous and heterozygous genes. Otherwise, by using primer for the CYP21 gene, only the CYP21 could be amplified, and most of time the CYP21/CYP21P chimeric gene will not be amplified. This will lead to a diagnostic discordance and result in false results. From our studies, we have shown that the lack of specific primer for this molecule missed the detection CYP21/CYP21P chimeric gene, which commonly occurs in the heterozygous CAH carrier population. Since the PCR amplification was a competition reaction, PCR dropout in detecting intron 2 nucleotides 656 of the chimeric gene was due to unequal concentration of the PCR template. Therefore, this PCR-based assay is a more effective way to analyze the mutation of CAH.

Program Nr: 2410 from the 1999 ASHG Annual Meeting

Molecular analysis in patients with MPS I: Two major common mutations and multiple single nucleotide polymorphic alleles in the IDUA gene. *P. Li, J.N. Thompson.* Department of Human Genetics, Univ. Alabama at Birmingham, Birmingham, AL.

Mucopolysaccharidosis type I (MPS I, MIM 252800) is an autosomal recessive disorder resulting from a deficiency of the lysosomal glycosidase, a-L-iduronidase (IDUA, EC 3.2.1.76). Patients with MPS I present with variable clinical manifestations ranging from severe type (Hurler syndrome), intermediate type (Hurler/Scheie syndrome), to mild type (Scheie syndrome). The clinical heterogeneity of MPS I has been suggested to reflect the allelic heterogeneity of the IDUA gene. In the present investigation, molecular analysis of the IDUA gene defects was performed for 10 patients who were enzymatically diagnosed with MPS I by the demonstration of IDUA deficiency. Total RNA and DNA samples were extracted from peripheral blood leukocytes or cultured fibroblasts. An experimental approach coupling RT-PCR based full-length cDNA sequencing with PCR based genomic DNA analysis was used for detecting mutations in the IDUA gene. Results of the analysis showed that one patient was a homozygote for a common mutation Q70X (CAG to TAG), one patient was a homozygote for another common mutation W402X (TGG to TAG), two patients were compound heterozygotes for Q70X/W402X, one patient was a compound heterozygote for Q70X and a novel missense mutation G208D (GGT to GAT), one patient was a compound heterozygote for R363C (CGC to TGC) and W402X, and one patient was a compound heterzygote for a novel missense mutation R162I (AGA to ATA) and a nonsense mutation R621X (CGA to TGA). The remaining three patients contained the W402X allele and an unidentified mutant allele. In addition, several single nucleotide polymorphic (SNP) alleles, coexisting with the disease-causing mutations, were detected. These SNP alleles included A8, A20, Q33H, L118, N181, A314, A361T, T388, and T410. The multiple IDUA SNP alleles could be useful for intragenic haplotyping in carrier detection and pedigree analysis. The present results and our previous data (Li. et al., Am J Hum Genet, 61:1490, Suppl.) indicated that the Q70X and W402X are found in about 25% and 40% of IDUA mutant alleles, respectively. Therefore, these two common mutations account for about 60% of mutant alleles in the IDUA gene.

Program Nr: 2411 from the 1999 ASHG Annual Meeting

Deficiencies in the Glucose-6-phosphate Transporter Cause Glycogen Storage Disease Type 1b but Not 1c. *B. Lin, H. Hiraiwa, C.-J. Pan, J.Y. Chou.* Heritable Disorders Branch, NICHD/NIH, Bethesda, MD.

Glycogen storage disease type 1 (GSD-1) is a group of autosomal recessive disorders caused by deficiencies in glucose-6-phosphatase (G6Pase) and the associated substrate/product transporters. Molecular genetic studies have demonstrated that GSD-1a and GSD-1b are defective in the G6Pase enzyme and a glucose-6-phosphate transporter (G6PT), respectively. While kinetic studies of G6Pase catalysis predict that the index GSD-1c patient is deficient in a pyrophosphate/phosphate transporter, the existence of a separate locus for GSD-1c remains unclear. We have previously shown that the G6Pase gene of the index GSD-1c patient is intact, we now show that the G6PT gene of this patient is normal. Further, we have uncovered five novel G6PT mutations among fourteen GSD-1b patients. We demonstrate that all fifteen missense G6PT mutations reported to date as well as the F93 and the prevalent 1211delCT G6PT mutations completely abolish or greatly reduce glucose-6-phosphate (G6P) transport activity. Our data establish the molecular basis of GSD-1b and strongly suggest the existence of a distinct GSD-1c locus.

Program Nr: 2412 from the 1999 ASHG Annual Meeting

Regulation of the human cystathionine beta-synthase (CBS) gene. *K.N. Maclean, M. Janosik, E. Kraus, J.P. Kraus.* Dept of Pediatrics, C-233,University of Colorado school of medicine , Denver, CO.

Cystathionine beta-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, while partial deficiency of CBS has been proposed to cause mild hyperhomocysteinemia, considered an independent risk factor for arteriosclerosis. The CBS gene has multiple transcription initiation sites with the 5' UTR being encoded by five alternatively used exons (-1a through -1e) and an invariably present exon 0. We have identified two separate promoters in the human CBS gene designated -1a and -1b, respectively. Using CBS activity assays it was found that the level of CBS activity in normal skin fibroblast, HepG2 (hepatoma) and SK-N-MC (neuroepithelioma) cells is coordinately regulated with proliferation. Maximal activity was detected during exponential growth and a 2 to 5-fold reduction of activity (depending upon cell type) occurred when cells became quiescent or were growth arrested in response to the addition of cytotoxic compounds or serum withdrawal. This finding was further investigated at the transcriptional level using -1a and -1b specific promoter constructs fused to a luciferase reporter gene and transiently transfected into HepG2 cells. In both cases, CBS promoter activity was coordinately regulated with proliferation as a function of nutrient depletion, growth rate, serum deprivation or the presence of cytotoxic compounds. A preliminary investigation has suggested that the second messenger compound cAMP is involved in the proliferation specific regulation of human CBS. The ability of insulin to repress CBS transcription from the -1b reporter construct, which lacks any known insulin response sequence elements, suggests that this regulation may be mediated by the intracellular concentration of cAMP. This hypothesis is supported by the observations that insulin specific repression of CBS was only detectable in actively proliferating cells and that the adenylate cyclase activator compound forskolin, up-regulates the -1b promoter in HepG2 cells.

Program Nr: 2413 from the 1999 ASHG Annual Meeting

Co-incidence of PKU and propionic acidemia in an Amish girl. *S.E. McCandless[1,3], J.W. McConnell[2], D.S. Kerr[2].* 1) Dept of Pediatrics, Univ of North Carolina; 2) Rainbow Babies and Children's Hosp, and; 3) Dept of Genetics, Univ Hospitals of Cleveland/Case Western Reserve Univ.

Propionic acidemia (PA) was recently diagnosed in an Amish female infant in whom PKU had been diagnosed by routine newborn screening (initial phe concentration 38 mg/dl). In addition to products of phe metabolism, methylcitrate and tiglylglycine were unexpectedly found in the urine organic acid analysis. Plasma and urine contained large amounts of propionylcarnitine. Enzyme analysis (fibroblasts) demonstrated very low propionyl-CoA carboxylase activity with normal activity of pyruvate and methylcrotonyl-CoA carboxylases. Urine pteridines were normal, as were serum biotinidase and RBC dihydropteridine reductase activities. At age 2 months, prior to treatment, she developed vomiting, decreased appetite and irritability, with metabolic acidosis and hyperammonemia (155mM). •• Management required combining two different amino-acid restricted formulas and oral carnitine (50 mg/kg/day). With treatment, phe was typically 2-6 mg/dl. At 6 months of age growth and development were normal. During this time of rapid growth the combination of the 2 formulas has met her requirements for essential amino acids without causing significant accumulation of phe or metabolites of propionyl-CoA. ••• There were several individuals with PKU on both sides of the family, but no recognized cases of PA nor consanguinity in the 4-generation pedigree. A 2-year-old sister of the proband with hypotonia and global developmental delay was tested and found to have PA but not PKU. That child had no episodes of serious illness, coma, blood dyscrasias or acidosis. •••• We are not aware of previous reports of co-incidence of these two rare metabolic diseases. This case alerts physicians to the possibility that rare genetic disorders may co-exist in a member of an in-bred group. The use of broader confirmatory testing enabled subclinical detection of PA in this case, and raises awareness of the usefulness of broader newborn screening, particularly in in-bred populations. The combined restriction of phe and propiogenic amino acids has been workable and effective, resulting in normal growth and development to date.

Program Nr: 2414 from the 1999 ASHG Annual Meeting

Immunolocalization Studies and Mutational Analysis of Acid a**-Glucosidase in Danon Disease.** *A.J. McVie-Wylie[1], P.W.K. Wong[2], S.G. Pophal[2], E.R. Rodriguez[3], V.J. Ferrans[4], D. van Leenen[5], A.J.J. Reuser[5], Y.T. Chen[1]*. 1) Dept. of Pediatrics, DUMC, Durham, NC; 2) Dept. of Pediatrics, RPSLMC, Chicago, IL; 3) Dept. of Pathology, RPSLMC, Chicago, IL; 4) Pathology Section, NBHLI, Bethesda, MD; 5) Dept. of Clinical Genetics, Erasmus University, Rotterdam, The Netherlands.

Glycogen storage disease type II (GSD II) is an autosomal recessive disease in which deficiency of lysosomal a-glucosidase (GAA) results in massive accumulation of glycogen. This disorder presents with a spectrum of clinical findings ranging from severe cardiomyopathy in infants to mild muscle weakness in adults. Danon disease resembles GSD II histopathologically, however, biochemical analysis indicates that GAA levels are within the normal range. This condition is characterized clinically by severe cardiomyopathy, mild myopathy and mild to moderate mental retardation. We describe a 5 year old male who mimics the clinical observations reported and has normal GAA activity in muscle and skin fibroblasts. The histological changes detected are consistent with previous findings, including faint perinuclear material within the myocardial muscle fibers, increased PAS staining in both skeletal and myocardial tissue, and intra-lysosomal and cytoplasmic glycogen accumulation. The biochemical defect in this disorder remains unknown. It has been postulated that the intracellular processing and routing of GAA may be defective. Human GAA is synthesized as a 110kDa precursor, which is subsequently cleaved during transport to the lysosomes. Western blot analysis indicates that our patient not only produces mature GAA but in quantities similar to normal controls, demonstrating normal GAA synthesis and processing. Immunolocalization staining in patient skin fibroblasts reveals a typical punctate pattern consistent with lysosomal localization. These data contradict the theory that abnormal GAA processing or misrouting may be responsible for this disorder. Furthermore, sequencing analysis revealed no deleterious mutations in the GAA cDNA coding region. We therefore conclude that GAA may not be integral in the pathomechanism of Danon disease and that other pathophysiologic changes may be critical in the etiology of this disorder.

Program Nr: 2415 from the 1999 ASHG Annual Meeting

Molecular characterization in Niemann-Pick type C: insights into phenotypic variablility. *V. Meiner[1], S. Shpitzen[2], M. Zeigler[1], A. Lossos[3], H. Mandel[4], G. Bach[1], E. Leitersdorf[2].* 1) Dept Genetics, Hadassah Univ Hosp, Jerusalem, Israel; 2) Center for Research, Prevention, and Treatment of Atherosclerosis, Hadassah Univ Hosp, Jerusalem, Israel; 3) Dept of Neurology, Hadassah Univ Hosp, Jerusalem, Israel; 4) Dept of Pediatrics, Rambam Medical Center, Haifa, Israel.

Niemann-Pick disease type C (NP-C) is an autosomal recessive lipid storage disease manifested by an impairment in cellular cholesterol homeostasis. The disease is clinically characterized by progressive degeneration of the central nervous system with accumulation of cholesterol and sphingomyelin. The clinical phenotype of NP-C is extremely variable ranging from an acute neonatal form to an adult-late onset presentation. To facilitate phenotype-genotype studies, we have analyzed multiple families with a clinical phenotype of NP-C. Clinical diagnosis in these families was supported by cholesterol esterification assays and filipin staining of cellular free cholesterol. The severity of the disease was assessed by age at onset of first symptoms, clinical progression, and cholesterol esterification studies. We screened for mutations in the entire NPC1 coding sequence and identified 2 novel mutations localized either within or between the putative transmembrane domains. A dinucleotide deletion predicted to result in a truncated 1120 aa protein was found in patients with a rapidly progressive fatal disease. In contrast, a novel missense mutation (A927V) was associated with an adult-onset, slowly progressive phenotype accompanied by a 23% to 58% block in the induced cholesterol esterification assays. These results, further support a possible link between the severity of the phenotype and the characteristics of the specific NPC1 mutation and may help to clarify the roles of putative critical domains in the NP-C protein.

Program Nr: 2416 from the 1999 ASHG Annual Meeting

Polyendocrinopathy, skeletal dysplasia, organomegaly, and distinctive facies associated with a novel, widely-expressed Gsa **mutation.** *K.A. Mockridge[1], M.A. Levine[2], L.A. Reed[1], E. Post[1], F.S. Kaplan[3], S.M. Jan de Beur[2], Z. Deng[2], C. Ding[2], C. Howard[1], R.E. Schnur[1]*. 1) Cooper Hosp./UMDNJ at Camden; 2) Johns Hopkins Univ. School of Medicine, Baltimore, MD; 3) Univ. of Pennsylvania School of Medicine, Philadelphia.

McCune Albright syndrome (MAS) is comprised of the clinical triad of polyostotic fibrous dysplasia, polyendocrinopathy, and café au lait macules. MAS is caused by somatic mosaicism for activating mutations of GNAS1, specifically at residues Arg201 and Gln227 in exons 8 and 9. We report a 2 yr old African American male with developmental delay and a variant MAS phenotype associated with a novel Gsa mutation that is apparently constitutional, rather than mosaic. Skeletal abnormalities include metaphyseal chondrodysplasia, with disproportionate, short limbs, bony effects of his hyperparathyroidism (demineralization, spontaneous fractures), slipped capital femoral epiphyses and advanced bone age. In addition, he has precocious puberty and increased testosterone, IGF-BP3, and somatomedin-C levels. He also has features suggestive of a storage disorder (hepatosplenomegaly, cardiomyopathy, thick skin and gums, increased urinary excretion of heparan and dermatan sulfate). Dysmorphic facial features include wide fontanel, epicanthi, hypertelorism, preauricular tags and sinuses, flat nasal bridge, short septum, tented upper lip, and deep philtrum. He has had therapy-resistant tinea versicolor, thrush, and monilial rash. He also has bilateral hydroceles. His cutaneous pigmentation is darker than either parent's, but he has no discrete café au lait macules. Because of the polyendocrinopathy, DNA was analyzed for mutations in the GNAS1 gene. A novel arginine to leucine mutation (CGT®CTT) was detected at residue 201. The mutation and the wild type allele appear to be present in equal proportions in DNA from both blood and fibroblasts and are equally expressed by RT-PCR of mRNA from these tissues. R201L is thus the first probably constitutional, non-mosaic GNAS1 mutation to be identified in association with a variant form of MAS. *GNAS1GNAS1GNAS1*.

Program Nr: 2417 from the 1999 ASHG Annual Meeting

I-Cell disease in a patient with unusual biochemical findings. *E.T. Morava Kozicz[1], G. Toth[1], E. Paschke[3], K.E. Jackson[2], Gy. Kosztolanyi[1]*. 1) Inst Medical Gen/Child Dev, Univ Medical Sch Pecs, Pecs, Hungary; 2) Human Genetics Program, Tulane University School of Medicine, New Orleans, LA; 3) Pediatric Hospital, Graz, Austria.

I-cell disease is a Hurler-like condition with coarse face, clear corneas, hyperplastic gums, severe growth deficiency, congenital hip dislocation, thoracic and vertebral deformities and restricted joint mobility. Retarded psychomotor development becomes evident in early infancy. We report a patient with typical clinical features of I-cell disease, who had unusual biochemical findings. Enzyme studies including arylsulfatase, hexosaminidase, iduronidase-sulfatase and beta-galactosidase showed normal activity in lysosomes, fibroblasts and serum. We found a pronounced decrease in the lysosomal activity of beta-glucoronidase (50%) with a 25 times increased activity in serum. Other metabolic findings including urinary excretion of sialic acid and no detectable GAG supported the diagnosis of I-cell disease. Since the mother is pregnant, prenatal diagnosis was performed, and no elevation of beta-glucoronidase or hexosaminidase A was found in the amniotic fluid, and the ratio of the enzyme activity in amniotic cells and culture media was comparable to controls.

Program Nr: 2418 from the 1999 ASHG Annual Meeting

Real defect of Ampd1 accompanied with myopathy: New missense mutations found in a Japanese patient. *T. Morisaki[1], H. Morisaki[1], I. Higuchi[2], M. Osame[2], M. Abe[3]*. 1) Dept Bioscience, Natl Cardiovasc Ctr Res Inst, Suita, Osaka, Japan; 2) Third Dept Medicine, Kagoshima Univ Med School, Kagoshima, Kagoshima, Japan; 3) Dept Medicine, Kagoshima Redcross Hosp, Kagoshima, Kagoshima, Japan.

AMPD (AMP deaminase) deficiency of skeletal muscle is one of the most common inherited defects in the Caucasian. Approximately 2 % of Caucasians are homozygotes for a nonsense mutation (C34T) in exon 2 of the AMPD1 gene. Alternative splicing of exon 2 in individuals who have inherited this defect is thought to provide a mechanism for phenotypic rescue and variations in clinical symptoms. Here we have studied the first case with real defect of AMPD1 gene in a Japanese patient with myopathy. Forty-six year-old female presented muscle weakness in lower extremities. The muscle biopsy showed negative staining for AMPD in addition to changes in fiber size and mild degeneration in myofiber. Muscle lysate showed very little AMPD activity (0.2 % of control). cDNA synthesized from the biopsied muscle indicated comparable amount of AMPD1 transcripts in the patient. Direct sequencing of cDNA identified two missense mutations in exon 9 and exon 10 of AMPD1. These mutations cause amino acid substitutions (R388W and R425H) in the conserved domain of AMPD1, and they were confirmed to be located in the different alleles by PCR analysis of genomic DNA. Prokaryotic expression showed comparable amount of AMPD1 peptide and undetectable AMPD activity in the constructs with these mutations. It has not been verified whether mutation of AMPD1 gene would indeed cause muscle dysfunction, since alternative splicing may restore the function of AMPD1 gene with C34T mutation. From this study, we conclude that this patient is a compound heterozygote for AMPD1 mutant allele and the first case of real dysfunction of AMPD1 gene product, suggesting that AMPD1 has indeed a key role in muscle metabolism and function.

Program Nr: 2419 from the 1999 ASHG Annual Meeting

Targeted disruption of the mouse iduronate sulfatase gene. *J. Muenzer, H. Fu.* Department of Pediatrics, University of North Carolina, Chapel Hill, NC.

Hunter syndrome (MPS II) is a lysosomal storage disorder due to a deficiency of the enzyme iduronate sulfatase (IdS). Deficiency of IdS results in lysosomal accumulation of dermatan and heparan sulfate, with progressive tissue and organ dysfunction, and in the severe form, premature death. A knockout mouse has been generated by homologous recombination. The targeting vector was designed to create a null mutation in the IdS gene. A replacement vector (JNS2) containing 9.2 kb of mouse genomic DNA was constructed in which a portion of exon 4 and all of exon 5 of the IdS gene (a 1.5 kb deletion) was replaced by a neomycin-resistance gene expression cassette. The targeting vector JNS2E8 was injected into E14TG2a embyronic stem cells and G-418 resistant clones were screened for homologous recombination. A targeting frequency of about 1:18 was obtained. Embyronic stem cells clones were injected into blastocysts that resulted in highly chimeric male mice which produced germline transmission of the mutated allele. Heterozygous female and hemizygous male mice were identified by Southern analysis and/or by a PCR assay which detects both the normal exon 4 and the recombinant exon 4 alleles. Offspring of heterozygous female mice yield the expected number of mutant male mice. The MPS II mice appeared normal at birth. The mutant male mice have no detectable IdS activity in liver or brain. Light microscopic analysis revealed vacuolated cells in liver and brain. Glycosaminoglycan content at 6 to 8 months of age was increased at least 2-fold in the seven tissues measured, with liver being greater than 6-fold elevated. Radiographs at 6 months of age revealed widened and thickened ribs. The IdS deficient mice should be useful in the study of the pathophysiology of Hunter syndrome and for the development of new therapies, such as enzyme replacement and gene therapy.

Program Nr: 2420 from the 1999 ASHG Annual Meeting

Difficult Prenatal Diagnosis of a Fetus Affected with Mild Smith-Lemli-Opitz syndrome (SLOS). *M.J.M. Nowaczyk[1,2], T.W. Heshka[2], L.E. Kratz[3], R.I. Kelley[3].* 1) Dept. Pathology & Molecular Medicine, McMaster University, Hamilton, ON, Canada; 2) Hamilton Regional Laboratory Medicine Program, Hamilton, Canada; 3) Kennedy Krieger Institute, Division of Metabolism, Baltimore, MD.

We recently diagnosed SLOS in two brothers minimal physical findings and mental retardation; their cholesterol levels were 2.30 and 2.34 mmol/l, and 7DHC levels were 134 and 183 mmol/l. We now present our experience with prenatal diagnosis of a third affected sib. The mother reported her pregnancy at 5 weeks of gestation, at which time oral cholesterol supplementation of 1000 mg/day was started. The couple underwent prenatal diagnosis by CVS at 11 2/7 weeks. The karyotype was 46,XY. Direct analysis of CVS material was performed according to previously published methods. The 7DHC/chol ratio was 3.76%, [normal controls: 0.06% ± 0.02% (SD), (n=7), unaffected at-risk: 0.08% ± 0.03% (SD)(n=7)]. This ratio was significantly lower than that found in CVS of three fetuses in whom SLOS was diagnosed prenatally [27.6% ± 4.5% (SD)] raising the possibility of a heterozygote fetus. The couple stopped the pregnancy at 13 2/7 weeks. The level of 7DHC in amniotic fluid obtained at that time was 151 ng/ml [normal value: 6 ± 3 (SD) ng/ml, (n=89)] and the 7DHC/chol ratio in fetal tissue was 69±37; [normal control 0.037±37;]. These results clearly established a diagnosis of SLOS.

This case illustrates the potential difficulties of interpreting results in fetuses with mild variants of SLOS. Although the 7DHC/chol ratio was »50 times normal, as it was much lower than the ratio in CVS tissue of other affected SLOS pregnancies, and because the cultured cells of some SLOS parents have had atypically high 7DHC/chol ratios, we could not be certain whether the intermediate value reflected heterozygote status or a fetus affected with mild SLOS variant. Also possible is that maternal chol supplementation for 6 weeks prior to CVS lowered the 7DHC/chol ratio in the CVS sample. As the clinical spectrum of SLOS expands to include cases with very mild biochemical abnormalities, the interpretation of the results of biochemical testing of fetuses affected with milder biochemical variants may be more difficult.

Program Nr: 2421 from the 1999 ASHG Annual Meeting

Glucosylsphingosine accumulation in patients with Gaucher disease. *E. Orvisky, E.I. Ginns, E. Sidransky.*
DHHS/PHS, NIH/NIMH, Bethesda, MD.

Gaucher disease results from the inherited deficiency of lysosomal glucocerebrosidase and presents with a wide spectrum of clinical manifestations including neuronopathic and non-neuronopathic forms. While patients and the null allele Gaucher mouse store the lipid glucocerebroside, elevated levels of a second and highly toxic substrate, glucosylsphingosine, have also been reported. Using high performance liquid chromotography with a 4-fluoro-7-nitrobenzofurazan-generated autofluorescent derivative, glucosylsphingosine levels were measured in tissues from patients with type 1, 2, and 3 Gaucher disease and correlated with patient phenotype. Glucosylsphingosine was measured in 13 spleen samples (5 type 1, 3 type 2, and 5 type 3) and levels ranged from 54 to 417ng/mg protein in the type 1 patients, 133 to 1200 ng/mg protein in the type 2 patients and 102 to 1298 ng/mg protein in the type 3 cases. The levels of glucosylsphingosine in these spleens bore no relation to the type of Gaucher disease, the age of the patient, the genotype, nor the clinical course; but tended to be highest in the largest and most abnormal spleens. In the same patients, glucosylsphingosine levels in liver were somewhat lower than those in spleen. Glucosylsphingosine was also measured in brains from 7 patients (1 type 1, 5 type 2, 1 type 3). While no elevation of glucosylsphingosine was found in the brain from the type 1 patient, the level in the type 3 patient was 8.89 ng/mg protein. The level in the type 2 patients ranged from 9.8 to 437 ng/mg protein, with the highest values detected in 2 fetuses with hydrops fetalis. The elevated levels found in brains from neuronopathic, but not type 1 Gaucher patients, support the hypothesis that glucosylsphingosine may contribute to the nervous system involvement in these patients.

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Program Nr: 2422 from the 1999 ASHG Annual Meeting

Compound heterozygosity for Fp subunit mutations causes complex II deficiency in a patient with encephalomyopathy. *B. Parfait, D. Chretien, A. Munnich, P. Rustin, A. Rotig.* INSERM U393, Hosp Necker, Paris, France.

Complex II of the mitochondrial respiratory chain is made up of four subunits in human. All four subunits are nuclearly encoded. The Fp (flavoprotein) gene is present in two copies on human genome, one on chromosome 5p15 and the second on chromosome 3q29 and the specific role of each copy remains unclear. We have sequenced the exon-intron boundaries of the two Fp copies by using somatic hybrids harboring either human chromosome 3 or chromosome 5. This allowed us to identify one base pair deletion creating a frameshift in the 3q29 copy and confirming that this gene is a pseudogene. Here, we report a patient with SDH deficiency presenting with encephalomyopathy. Sequencing the Fp SDH cDNA allowed to identify two heterozygous mutations. One allele carried a C to T transition changing an alanine into a valine in the protein. This transition was found in the patient's father but was absent from 150 controls. Transfection of the mutant cDNA in human SDH-deficient cells failed to restore normal SDH activity confirming the deleterious nature of this mutation. The second allele, inherited from the mother, carried an A to C substitution changing the methionine translation initiation codon into a leucine. The mutated transcript represented only 20% of total Fp transcripts suggesting instability of this transcript. This new case of SDH Fp mutation adds further support to the view that nuclear genes are involved in respiratory chain deficiencies in human.

Program Nr: 2423 from the 1999 ASHG Annual Meeting

Regulated expression of the mouse acid b **glucosidase (GCase) gene.** *E. Ponce[1], A. Hung[2], D. Witte[1], G.A. Grabowski[1]*. 1) Children's Hospital Medical Center, Cincinnati, OH; 2) Escuela de Medicina J.M. Vargas UCV, Caracas, Venezuela.

The hydrolysis of glucosylceramide to ceramide and glucose occurs by the action of the lysosomal enzyme GCase. In Gaucher disease, an autosomal recessive inherited disorder, the deficiency of this enzyme results in the accumulation of glucocerebrosides in spleen, liver and bone marrow and CNS manifestations in severe variants. Although GCase activity is ubiquitously distributed in mammalian tissues, the pattern of mRNA expression has not been reported. *In situ* hybridization studies of mouse early postnatal (2, 4 days old) and adult tissues were conducted to evaluate the spectrum of GCase mRNA expression. mRNA signals were present in all tissues and cell types examined, consistent with the distribution of GCase activity. Distinct patterns of differential expression were identified in specific tissues, cell types, and developmental stages. In the CNS, low intensity signals evenly distributed between neurons and glia at early postnatal times, were much lower in adult tissues. The highest expressing cells were in the GI tract. Intense signals were localized to the forestomach and esophagus squamous epithelia and intestinal cell lining. In the liver, the greatest signals were present in hepatocytes. Discrete signals were present in the capsule of lymph nodes and cells lining the sinusoidal spaces. The highest intensity signals in the spleen were in cells of the germinal centers. The thymus had diffuse signal. In the kidney, the signals were localized to the cortex, nephrogenic zone and immature tubules cells while in the lungs the cell involvement was generalized. Distinct low intensity signals were evident in Sertoli cells of the testis. Skeletal and cardiac muscle cells had very low level signals. Interestingly, the bone showed no detectable signal in hematopoietic cells but distinct low intensity signal was present in cells of the young trabeculae within the cartilage to bone conversion zone. These results show tissue, cell and developmental stage specific regulation of the GCase locus at the transcriptional level with no direct correlation to the tissue specific manifestations of Gaucher disease.

Program Nr: 2424 from the 1999 ASHG Annual Meeting

Early phenotyping in phenylketonuria by genotyping and screening procedures. *A. Ponzone[1], O. Guardamagna[1], S. Ferraris[1], G.B. Ferrero[1], C. Carbonara[1], R. Ponzone[2], S. Giannattasio[3], M. Spada[1]*. 1) Dipartimento di Pediatria, Univ. di Torino, Torino, Italia; 2) Dipartimento Scienze Ostetriche e Ginecologiche, Univ. Torino; 3) Centro di Studio su Mitocondri e Metabolismo Energetico, Bari, Italy.

The clinical spectrum of phenylketonuria (PKU) encompasses a continuum of phenotypes with different severity, which have been separated into four arbitrary classes of phenylalanine hydroxylase (PAH) deficiency: severe, moderate, mild, and benign. As early patient phenotyping is of crucial interest for the immediate therapeutic option as well as for the long-term prognosis, we report the informations drawn from the study of 26 PKU patients, all genotyped for their causal mutations at the PAH locus. They were analyzed in the neonatal period and subsequently re-evaluated for several biochemical parameters: blood phenylalanine (Phe) level at the screening test, pre-treatment plasma Phe concentration and Phe/tyrosine ratio, velocity of plasma Phe normalization after a Phe-free diet was started, maximal and minimal dietary Phe tolerance. A total of 20 PKU mutations were detected in the 52 chromosomes, of which 12 were known as null, 4 as mild, and 4 as benign. On the basis of their allelic combination, 11 patient were assigned to the severe, 4 to the moderate, 6 to the mild, and 5 to the benign class of PAH deficiency. Within the examined metabolic parameters, only the value of dietary Phe tolerance, both maximal and minimal , showed a precise correlation with the predicted and observed phenotype. Contrary to previous opinion, the values of blood Phe concentration at the initial screening test and at the pre-treatment time, though reflecting allelic differences at the PAH locus, were not fully predictive of the inherent phenotype, sharing consistent overlap with one or more of the adjacent classes. Since the value of minimal dietary Phe tolerance can be assessed in patients as early as by the first month of life, it allows to anticipate a clinic-based and reliable PKU phenotyping.

Program Nr: 2425 from the 1999 ASHG Annual Meeting

Long term follow up study and linkage analysis of hepatic Carnitine Palmitoyl Transferase-1 (CPT1A) deficiency in a Canadian and American Hutterite kindred: evidence for a founder effect. *C. Prasad[1], L. Dilling[1], M. Innes[1], N. Buist[2], O. Hamilton[3], L. Beischel[3], J.P. Johnson[3]*. 1) Dept. of Genetics, Childrens Hosp, Winnipeg, MB, Canada; 2) OHSU, Portland, OR, USA; 3) Shodair Hospital, Helena, MT, USA.

CPT1A deficiency is a rare autosomal recessive defect of mitochondrial beta-oxidation presenting with hypoketotic hypoglycemia, hepatomegaly, seizures, and coma. We describe 5 patients with CPT1A deficiency who are all members of the Hutterite Brethren, 3 of whom have been reported previously (Haworth et al., J. Pediatr 1992). All presented with recurrent episodes of encephalopathy associated with hypoglycemia, hyperammonemia, and hepatomegaly. Diagnosis was confirmed by low enzyme activity (CPT1A) in cultured skin fibroblasts. Extended follow up shows that both index patients have ongoing seizures and developmental delay as consequences of delayed diagnosis and hypoglycemic brain injury. The patients in whom the diagnosis was suspected earlier show normal neurodevelopmental progress. The Canadian patients belong to an extended inbred Hutterite family (a brother, sister and their second cousin), while the American patients are sibs. Linkage analysis was undertaken in the American family. We used 7 polymorphic DNA markers near CPT1A on chromosome 11q13, including D11S987, reported to be within 1 Mb of the gene (Britton et al., Genomics 1997). Results showed the 2 sibs were homozygous for a haplotype of 5 loci over approximately 10 cM. This haplotype was present in both grandfathers, who were first cousins. This indicates a common ancestor in the fifth generation preceding the affected sibs. A founder effect was further confirmed by finding homozygosity for haplotypes identical at 4 of the 5 loci in the 3 Canadian patients. The Canadian and American Hutterite families share a common ancestor around 1812, about 60 years before the Hutterites arrived in North America. Mutation analysis on the Canadian patients has been carried out and is presented in a separate submission. Given the presumed identity by descent of the CPT1A mutations in these two families, we expect identical mutations in both.

Program Nr: 2426 from the 1999 ASHG Annual Meeting

The ALD-Related gene: of functional redundancy and mouse model for adrenoleukodystrophy. *A. Pujol, E. Metzger, N. Troffer-Charlier, C. Kretz, J.L. Mandel.* Human Molecular Genetics, IGBMC, Strasbourg, France.

X-linked adrenoleukodystrophy (X-ALD), is a severe demyelinating disease associated with impaired beta-oxidation of very-long-chain fatty acids. The gene responsible for the disorder encodes a peroxisomal half-ABC transporter, the ALD protein. ALD is characterized by a very high phenotypic variability within the same kindred, a fact that suggests the existence of modifier genes. An animal model of the disease is not available yet, since ALD knock-out (KO) mice do indeed show a biochemical phenotype (accumulation in organs of VLCFAs) but no demyelination. We have characterised the human ALDR gene structure, the closest homolog to the ALD gene (66% identity at the aa level). The ALDR gene has been shown to complement the biochemical phenotype of X-ALD patients fibroblasts [1]. We aim at demonstrating the in vivo capacity of the ALDR gene for functional rescue of the ALD KO mouse's biochemical phenotype. Therefore we have constructed transgenic mice expressing the mALDR cDNA under the control of an ubiquitous promoter (chicken beta actin); a line showing high expression of the transgene in several tissues has been obtained and crossed with ALD KO mice. VLCFA's levels and beta-oxidation capacity of these mice will be determined. Furthermore we believe on the hypothesis of some functional redundancy between the ALD and ALDR genes, and intend the creation of a mouse model of the disease by generating a double ALD/ALDR KO. For the ALDR KO, we have disrupted the ALDR gene in the mouse, and found that homozygous mice (ALDR-/-) are not viable. Analysis of ALDR KO embrios will help to gain insight into the function of the ALDR gene, and possibly into the issue of functional redundancy at the peroxisomal membrane. [1] Kemp et al, Nature Med 4, (1998), 1261.

Program Nr: 2427 from the 1999 ASHG Annual Meeting

Cystinuria type I: Identification of nine new mutations by RNA-SSCP and two large deletions by multiplex QF-PCR in *SLC3A1*. *J. Purroy[1], L. Bisceglia[2], L. Feliubadaló[1,3], F. Rousaud[4], L. Zelante[2], A. Zorzano[3], X. Estivill[1], M. Palacín[3], P. Gasparini[2], V. Nunes[1]*. 1) Centre de Genètica Mèdica i Molecular-IRO, Barcelona, Catalonia, Spain; 2) SGM-Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy; 3) Departament de Bioquímica i Fisiologia, Facultat de Biologia, UB, Barcelona, Catalonia, Spain; 4) Servei de Nefrologia, Fundació Puigvert, Barcelona, Catalonia, Spain.

Cystinuria type I is caused by mutations in *SLC3A1*, although only half of the patients studied show mutations in this gene. Here we describe nine new mutations in *SLC3A1* (S168X, M190T, 765+1G>T, F266S, R452Q, Y461X, S547W, L564F and C673W) detected after studying 38 chromosomes (24 type I and 14 from non fully determined patients) by RNA-SSCP. To detect large deletions we set up a method combining multiplex and semi-quantitative fluorescent PCR. By a simultaneous amplification of the 10 exons of *SLC3A1* we detected two large deletions in a Belgian family: one comprising exon 10 and another comprising exons 2 to 10 and beyond. We have found mutations in 60% (23 out of 38) of the chromosomes studied. These results are consistent with the 60% of cystinuria type I chromosomes that have been explained so far in different studies by mutations in *SLC3A1*. The large number of non-explained chromosomes may be accounted for by the existence of mutations in the promoter or other less studied areas, by the difficulty of distinguishing between type I and type III phenotypes or by the existence of a second gene for cystinuria type I.

Program Nr: 2428 from the 1999 ASHG Annual Meeting

Quantitative amino acid analysis using a Beckman System Gold HPLC 126AA analyzer. *Y. Qu[1], R.H. Slocum[2], W.E. Rasmussen[1], N.J. Carpenter[1], J.G. Coldwell[3], B. Say[1].* 1) H A Chapman Inst Med Genetics, Tulsa, OK; 2) Eltanin Inc., San Francisco, CA; 3) Children's Medical Center, Tulsa, OK.

Beckman System 6300 and 7300 Amino Acid Analyzers are no longer available on the market. To develop a quantitative amino acid analysis program for diagnosis and management of patients with amino acidemias, a Beckman System Gold HPLC ion-exchange system was used. The analysis system includes Solvent Module 126 AA, Post Column Reactor 232, Detector 166, Autosampler 507, and a 3mm X 250mm spherogel AA Lithium column. The Lithium column eluents and Trione Nihydrin Reagent were from Pickering Laboratories.

In the first stage, a 30-min isothermal, isocratic HPLC chromatograph was developed for quantitative analysis of valine (val), methionine (met), isoleucine (ile), leucine (leu), phenylalanine (phe) and tyrosine (tyr) using both plasma and dried blood spots. The quantitation of met, leu, and phe was validated by the Centers for Disease Control Newborn Screening quality assurance program from dried blood spots. This short program was successfully used for confirmatory testing for hyperphenylalaninemia patients from State Newborn Screening program. In addition, the program was used to monitor dietary management for phenylketonuria and tyrosinemia type 2 patients. It also has the potential to be used for diagnosis and management of patients with maple syrup urine disease and homocystinuria. The second stage of development involved screening 42 amino acids from phosphoserine to arginine. The separation was achieved by using three temperatures and a step gradient with three Pickering Lithium eluents. The chromatogram was comparable to that obtained from a Beckman System 6300 Amino Acid Analyzer. At 500 nm wavelength, all primary amino acids and hydroxyproline and proline were separated and detected in a single run. This running system provides a less expensive and reliable way to analyze amino acids for patients with amino acidopathies.

Program Nr: 2429 from the 1999 ASHG Annual Meeting

Functional implications of intramolecular alterations on human galactocerebrosidase. *M.A. Rafi, H.Z. Rao, P. Luzi, D.A. Wenger.* Dept Neurology, Thomas Jefferson Univ, Philadelphia, PA.

Galactocerebrosidase (GALC) is the enzyme responsible for lysosomal degradation of galactosylceramide and lack of GALC activity results in Krabbe disease. Its expression in normal cells is governed by a weak promoter, and sub-optimal initiation sequences. This results in a small but sufficient amount GALC in normal cells. The enzyme is very hydrophobic, with strong tendency to aggregate in a very high molecular weight complex. These biochemical and biological peculiarities render the purification of GALC extremely difficult. However generating sufficient amount of this enzyme is critical to obtain a polyclonal antibody, which in turn is pivotal to investigate many physiologic processes including enzyme processing. We designed a wide range of plasmid constructs, to increase the yield of recombinant GALC, and facilitate its intracellular detection. Alterations introduced in our pcDNA3-GALC construct include: addition of sequences for green fluorescent protein (GFP) at the 3'end, tagging the protein with a short stretch of peptide containing a polyhistidine tract, entrokinase specific cleavage site, and c-myc epitope at N- and C-terminals of GALC, and finally replacing original leader peptide with leader sequence for mouse Ig kappa chain. The GALC activities were then evaluated in COS-1 cells. Low GALC activity was detected when GFP was fused at its C-terminal but normal activity was restored when an IRBS was introduced in the construct between coding sequences for GALC and GFP. GALC tagged with short segment of peptide containing the polyhistidine tract, entrokinase specific cleavage site, and c-myc epitope preserved its activity when this segment was located at either the N- or C-terminal of the GALC. A construct with the leader sequence from mouse Ig kappa chain failed to increase GALC secretion, but surprisingly demonstrated slightly higher intracellular GALC activity compare to one with the original leader sequence. These findings are leading to experiments using retroviral vectors where the overproduced GALC could be easily purified using a nickel affinity column followed by release of GALC by treatment with entrokinase.

Program Nr: 2430 from the 1999 ASHG Annual Meeting

Characterization of the gene encoding a**-aminoadipic semialdehyde synthase.** *K.A. Sacksteder[1], B.J. Biery[2], J.C. Morrell[1], B.V. Geisbrecht[1], R.P. Cox[3], S.J. Gould[1], M.T. Geraghty[2].* 1) Dept Biological Chemistry, Johns Hopkins Univ, Baltimore, MD; 2) Dept. Pediatrics, Johns Hopkins Univ, Baltimore, MD; 3) Univ. of Texas Southwestern Medical Center, Dallas, Texas.

A-aminoadipic semialdehyde synthase is a bifunctional enzyme which catalyzes the conversion of lysine to a-aminoadipic acid. The enzyme is associated with both lysine-ketoglutarate reductase and saccharopine dehydrogenase activities. Defects in the bifunctional protein result in familial hyperlysinemia, an autosomal recessive condition characterized by hyperlysinemia, lysinuria and variable saccharopinuria. We used sequences from *S. cerevisiae*, namely the LYS1 gene which encodes saccharopine dehydrogenase activity and the LYS9 gene which encodes lysine-ketoglutarate reductase activity to search the EST database and subsequently cloned a full length cDNA encoding the bifunctional enzyme. The cDNA contains an ORF of 2781 bp predicted to encode 927 amino acids. The cDNA is arranged such that the 5' end is homologous to the yeast saccharopine dehydrogenase, while the 3' end of the cDNA is similar to the yeast lysine-ketoglutarate reductase. In Northern blot analysis the cDNA crosshybridizes to several transcripts. These are most highly expressed in liver and to a lesser extent in most other tissues. The genomic structure consist of 24 exons scattered over 68 kb and maps to chromosome 7q 31-32. We sequenced the genomic DNA from a patient (JJa) who has been previously reported with familial hyperlysinemia and who is the product of a consanguineous mating. The patient has a homozygous out of frame 9 bp deletion in exon 15 which results in a premature stop codon at position 534 of the protein.

Program Nr: 2431 from the 1999 ASHG Annual Meeting

Type IV 3-methylglutaconic aciduria associated with isolated complex II and IV deficiencies . *F. Scaglia[1], V.R. Sutton[1], H. Vogel[2], S.K. Shapira[1].* 1) Depts. of Molecular and Human Genetics; 2) and Pathology, Baylor College of Medicine, Houston, TX.

Type IV 3-methylglutaconic aciduria (Mc Kusik 250951) has been described in children who present during the first year of life with hypotonia, developmental delay, seizures, cerebellar dysgenesis, retinal pathology, hypertrophic cardiomyopathy, hepatic dysfunction, and dysmorphic features. Some of these patients have had complex I, IV, and/or V deficiencies, or Pearson syndrome, suggesting that 3-methylglutaconic aciduria may be associated with mitochondrial dysfunction. Furthermore some cases have been associated with mitochondrial DNA depletion. We report a three year old boy who lost developmental milestones after four months of age. His presentation revealed psychomotor retardation, dystonic movements, and hypotonia, prompting a genetic evaluation. Urine organic acids analysis revealed large amounts of glutaric acid, 3-hydroxyglutaric acid, and glutaconic acid, suggesting the diagnosis of glutaric acidemia type I. Enzyme assay for glutaryl-CoA dehydrogenase was normal. Additional studies were performed, excluding glutaric acidemia type II. While on treatment with carnitine and riboflavin, urine organic acids analyses showed a different pattern from initial studies, with large amounts of 3-methylglutaconic and 3-methylglutaric acids. Assay for 3-methylglutaconyl-CoA hydratase was normal. On muscle biopsy the electron microscopy showed Z-disc streaming, and abnormal cristal configurations within the mitochondria. Respiratory chain enzyme analysis showed partial deficiency of complex II and IV activities. Evaluation for a possible mitochondrial DNA depletion is currently underway. This is the first report of a patient with 3-methylglutaconic aciduria associated with isolated complex II and IV deficiencies. The 3-methylglutaconic aciduria observed in this patient most likely represents a secondary phenomenon, and it is unlikely to be the cause of the clinical phenotype. However it appears to be a useful indicator of an underlying defect in the respiratory chain, which should prompt future studies toward elucidating the molecular cause of type IV 3-methylglutaconic aciduria.

Program Nr: 2432 from the 1999 ASHG Annual Meeting

Genomic organisation of the human phosphomannose-isomerase (PMI) gene and mutation analysis in patients with the carbohydrate-deficient glycoprotein syndrome type Ib (CDGS1b). *E. SCHOLLEN[1], L. DORLAND[2], J.B.C. DE KLERK[3], O.P. VAN DIGGELEN[3], G. MATTHIJS[1]*. 1) Center for Human Genetics, University of Leuven, Leuven, Belgium; 2) Wilhelmina Kinderziekenhuis, Utrecht, Nederland; 3) Academic Hospital Rotterdam, Rotterdam, Nederland.

The carbohydrate-deficient glycoprotein (CDGS) syndromes are a group of multi-system disorders, characterised by incomplete glycosylation of glycoproteins. Two subtypes relate to defects in the synthesis of GDP-mannose, in the earliest steps of N-glycosylation: a deficiency of phosphomannomutase in type Ia (CDGS1a) and a deficiency of phosphomanno-isomerase in the recently discovered type Ib (CDGS1b). CDGS1b patients present with intestinal symptoms, including protein-losing enteropathy and liver disease, sometimes with fatal outcome, and coagulation problems. We determined the genomic structure of the phosphomannose-isomerase (PMI) gene, to allow mutation analysis at the genomic level and to prepare for screening. The human PMI gene consists of 8 exons and spans 7 kb, in contrast to the mouse gene that comprises only 6 exons. Two patients in two consanguineous families were found to be homozygous respectively for the M51T and R152Q mutation. In a third patient, only one mutation (R152Q) was found; the second mutation must be located outside the coding region. This is reminiscent of a prior case, in which a second mutation could not be found, in spite of a biochemically proven carrier-status in both parents. We are currently focussing on the promoter sequence of the gene in an attempt to identify regulatory mutations. The disease is treatable with mannose, and therapy has been started in these patients. Because the disease seems to be largely unrecognized, it is important to also try and raise awareness on this disease.

Program Nr: 2433 from the 1999 ASHG Annual Meeting

Short Chain Acyl-CoA Dehydrogenase Deficiency (SCADD) - An Under-Reported Disorder? *P.H. Schwartz, S.A. Stein.* Neurology and Brain/Tissue Bank Dev Disorder, Children's Hosp Orange County, Orange, CA.

SCAD, an intramitochondrial enzyme of the beta-oxidation spiral, catalyzes the oxidation of 6- and 4-carbon acyl-CoA fatty acid esters to release electrons and give rise to the corresponding 2-trans-enoyl-CoA fatty acid esters. The clinical presentations and laboratory findings of patients with deficiencies of SCAD are highly variable and may include hypoketotic hypoglycemia, myopathy with or without myoglobinuria, elevations in lactate (L), butyrate (Bu), ethylmalonate (EtM), or methylsuccinate (MeS), and low plasma or muscle carnitine. Post-mortem/biopsy findings have revealed hepatosplenomegaly with fatty changes or minor generalized lipid accumulation in type I myofibers. We present four patients with enzymatically-verified SCADD whose findings further broaden the phenotypes of SCADD. The salient features of each patient are: Patient 1: severe neonatal weakness/floppiness, mild cardiomegaly, and glaucoma; mild elevation of EtM and Kreb's cycle intermediates; numerous intramyofibrillar lipid droplets with increased size and number of mitochondria. Patient 2: delayed language, facial weakness, slow motor and intellectual development; mild elevations of L, EtM; slightly low plasma carnitine; normal muscle biopsy findings. Patient 3: neonatal weakness and hypotonia, mild dysmorphism; normal development; low plasma carnitine; type II myofiber size disproportion; mild increase in EtM, and MeS. Patient 4: normal birth and development; severe cerebral edema after RSV infection and SVT; normal pathological and laboratory findings. All four of these cases were tentatively diagnosed because of a high index of suspicion using the Fatty Acid Oxidation Defects screen of Charles Roe at Baylor. Only in Patient 1 were the clinical and laboratory findings relatively consistent with what is felt to be the salient findings in disorders of fatty acid metabolism; the remainder of the patients represent new phenotypes of SCADD. This indicates that SCADD may be an under-reported genetic disease and may not have any clinically observable symptoms. SCADD, like other metabolic disorders such as GA I, may only become apparent under metabolically demanding circumstances.

Program Nr: 2434 from the 1999 ASHG Annual Meeting

Detection of enzyme deficiencies by Electrospray Ionization Mass Spectroscopy (ES/MS). *C.R. Scott, S. Gerber, F. Turecek, M. Gelb.* Depts of Chemistry and Pediatrics, Univ Washington, Seattle, WA.

We have developed a sensitive, specific, rapid and general approach for measuring enzyme activity in crude cell lysates using ES/MS as the detection system. The strategy consists of synthesizing substrates consisting of biotin-sarcosine-polyethylene glycol-glycoside. Biotin is linked to sarcosine to provide an affinity handle for selective capture and simple purification by streptavidin-agarose. Sarcosinyl biotin forms an N-methyl biotinamide motif that blocks cleavage by biotinidase and affords rapid elution from streptavidin. The PEG diamine linker provides water solubility, contains a basic residue for protonation by electrospray (high sensitivity), allows for conjugate differentiation of coincident mass by insertion or deletion of a PEG unit, and provides a location for introduction of stable isotopes for synthesis of internal standards. Finally, the PEG linker is coupled to a clinically revelant substrate to form the complete substrate conjugate. Following incubation with cell lysates the substrate and product are captured and then seperated and quantitated by ES/MS. We have applied the method to identifying disorders of lysosomal storage and have verified deficiency of B-galactosidase and N-acetylglucosaminidase in patients with GM1-gangiosidosis and Sanfillipo syndrome, respectively. The method can measure more than one enzyme reaction simultaneously (multiplexing) and can be developed to identify the enzyme deficiency of a disorder that has a common phenotype, but caused by different enzyme deficiencies.

Program Nr: 2435 from the 1999 ASHG Annual Meeting

Diagnosis of familial juvenile hyperuricaemic nephropathy. *I. Sebesta, B. Stiburkova, S. Kmoch.*
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Hyperuricaemia and/or gout are uncommon in children and adolescents. Familial juvenile hyperuricaemic nephropathy (FJHN) is a disorder characterized by progressive renal disease affecting young men, women and children equally. Gout is not a constant feature. The most striking finding is progressive renal disease with early onset. This presentation distinguishes FJHN from the more prevalent primary gout. The biochemical hallmark is hyperuricaemia arising from a reduced excretion fraction of uric acid. Autosomal mode of inheritance was observed, but the gene(s) responsible for the renal impairment is (are) still unknown. The diagnosis depends on clinical and biochemical evaluation. Complete laboratory investigations were set up. These include: evaluation of uric acid in plasma and especially in urine, quantification of purine metabolites in urine and assays of purine enzymes: hypoxanthine phosphoribosyltransferase (HPRT) and phosphoribosylpyrophosphate synthetase (PRPPs). The concentrations of urinary uric acid were expressed on creatinine and creatinine clearance basis. More than 400 patients with unexplained hyperuricaemia were investigated and 12 new patients with FJHN spanning two and three generations in two Czech families were found. Detailed studies of the relatives in the previously described family revealed four additional patients. The youngest patient was 6-year-old girl and six other patients were under the age of twenty. The patients suffered from nonspecific interstitial nephropathy. Three young patients have mildly impaired renal function. Our patients show the evidence of early onset of this disorder. Moreover,we found that the detailed purine investigations are essential for the exclusion of hyperuricaemia due to defect of HPRT or PRPPs, particularly in older patients with renal impairment. The need for DNA marker for presymptomatic detection is of prime importance. Taking to account our and other described patients with FJHN the indications for purine metabolic work up are: unexplained hyperuricaemia in children or young adults found within family members.(Supported by grant 27-3 Ministry of Health,CZ; EC grant ERBIC20CT98-0212).

Program Nr: 2436 from the 1999 ASHG Annual Meeting

Cloning, expression and characterization of human guanine deaminase (GDA) and identification of the *E. coli* homolog. *F.F. Snyder, R.G. Yuan, W.J. Paramchuk, J.C. Bin, J.T. Maynes.* Department of Medical Genetics, University of Calgary, Calgary, Alberta, T2N 4N1, Canada.

Guanine deaminase is an aminohydrolase that converts guanine to xanthine and ammonia. The reaction removes the guanine base from the pool of guanine containing metabolites and may play a role in the regulation of cellular GTP. We purified the mouse guanine deaminase protein to homogeneity and obtained partial amino acid sequence. BLAST analysis facilitated the retrieval of human EST clones. We subsequently cloned and sequenced the cDNA for human brain guanine deaminase, having an open reading frame of 1365 nucleotides and encoding a 51.0 kDa protein. The corresponding homolog in the *E. coli* genome was identified at 65.2 minutes as a functionally unassigned 1317 nucleotide open reading frame encoding a 50.2 kDa protein that is 36% identical to the human enzyme. Expression and purification of the recombinant proteins gave K_m's for the human and *E. coli* enzymes of 9.5 and 18 mM respectively. The enzymes share a 9 residue motif, PG[FL]VDTHIH, characterised as a class II zinc site, with no less than 10 other amino- and amido-hydrolases. The human and mouse motifs are identical with L at the variable position versus F for the *E. coli* enzyme. Atomic absoprtion demonstrated that both the human and *E. coli* enzymes have approximately 1 atom of Zn per guanine deaminase subunit and the activity was abolished by exposure to the divalent metal ion chelating agent, phenanthroline. Analysis of tissue expression shows high levels of guanine deaminase mRNA in the brain, liver, kidney and digestive tract. There is essentially no expression in cardiac tissue, skeletal muscle, reproductive or lymphoid systems. The pattern of expression in the brain was complex with expression in the cerebral cortex, frontal, parietal and occipital lobes, and maximal levels in the temporal lobe and amygdala. Southern analysis reveals the human guanine deaminase gene to span approximately 33 Kb. Supported by the Medical Research Council of Canada.

Program Nr: 2437 from the 1999 ASHG Annual Meeting

“Age related” Stargardt disease accounted for by heterozygote truncating mutations in the ABCR gene questiones autosomal recessive inheritance. *E. Souied[1, 2], J.-M. Rozet[1], D. Ducroq[1], S. Gerber[1], I. Perrault[1], G. Coscas[2], G. Soubrane[2], A. Munnich[1], J. Kaplan[1]*. 1) Unité de Recherches sur les Handicaps Génétiques de l'Enfant, INSERM U393, Hôpital Necker, Paris, France; 2) Clinique Ophtalmologique Universitaire, Centre Hospitalier Intercommunal, Créteil, France.

Stargardt disease (STGD) and late-onset *fundus flavimaculatus* (FFM) are autosomal recessive macular dystrophies with perimacular flecks of childhood and adulthood respectively. The STGD and the FFM genes have been independently mapped to 1p22.1 and mutations of the ATP binding cassette retinal specific (ABCR) have been shown to account for both phenotypes. In addition, heterozygous mutations of the ABCR gene have been identified in patients with age related macular dystrophies (ARMD), raising the hypothesis that the ABCR gene acts as a susceptibility factor in this condition. We report here the ABCR gene analysis of two unrelated patients affected with “age related” Stargardt disease with an onset at 70 and 65 years of age respectively. For this reason, the patients could have been misdiagnosed as ARMD. Nevertheless, both displayed typical angiographic features of Stargardt disease, namely macular atrophy, perimacular fluorescent flecks and silent choroid. The ABCR gene was entirely analyzed by direct sequencing in the two patients. In the first one, an heterozygote C to T transition at nucleotide 450 was identified resulting in the substitution of an arginine to a stop codon at position 152. In the second, an heterozygote splice mutation at the acceptor site of exon 35 (4849-1 G®C) was identified. The 49 other exons and the promoter region of both patients were extensively sequenced, but no deleterious base change has been detected on the second allele. Although we cannot totally exclude an overlooked mutation in the two patients, our results strongly suggest that this late-onset phenotype is accounted for by heterozygous truncating mutations in the ABCR gene and opens the debate of inheritance patterns of the ophthalmologic disorders related to the ABCR gene.

Program Nr: 2438 from the 1999 ASHG Annual Meeting

Investigating the pathogenesis of mucolipidosis IV using subtractive hybridization. *M. Sun, R. Schiffmann, E. Goldin.* Developmental and Metabolic Neurology Branch, NINDS, NIH, Bethesda, MD.

Mucolipidosis IV is an autosomal recessive metabolic disorder affecting the development of motor and communicational functions. Progressive loss of vision is evident in most patients. Vacuolization of certain epithelial cells, neurons, and fibroblasts is manifested. The gene mutation and the cause of the apparent neurodevelopmental arrest are not known. To better understand the defects underlying the cellular pathology in mucolipidosis IV we analyzed gene expression using subtractive hybridization. Fibroblast cDNA was prepared from purified mRNA and subtractive library was created using PCR-Select subtraction kit from Clontech. We subtracted RNA of normal fibroblasts from that of patients, and vice versa. The initial subtraction resulted in over 8000 colonies in the patient over-expressed library, and 3000 in the under-expressed library. The clones obtained from these subtractions were then screened against the products of subtraction of 3 other patient and normal cultures. Over 500 clones of genes over-expressed in patients and100 clones of genes under-expressed in patients were sequenced. Among the over-expressed genes, fibronectin was the most prominent, appearing in 200 clones. Other over-expressed genes were also part of the extracellular matrix or genes involved in cellular stress. Several over-expressed genes were related to vesicular transport, which taken together with vacuolization of cells, suggest that abnormal vesicular transport pathways play an important role in the pathogenesis of mucolipidosis IV. In the group of under-expressed genes the most prominent was insulin-like growth factor binding protein 3 (IGFBP3) appearing in 14 clones. Other genes in this group included integrin related proteins and stress related genes. The fact that over-expressed and under-expressed gene populations were substantially different adds to the credibility of technique used in our experiments. Overall, the changes in gene expression in cultured mucolipidosis IV fibroblasts serves as a reliable indicator for the cellular pathology in this devastating disorder.

Program Nr: 2439 from the 1999 ASHG Annual Meeting

Relationship between kinetic properties of mutant enzyme and biochemical and clinical responsiveness to biotin in holocarboxylase synthetase deficiency. *Y. Suzuki[1], O. Sakamoto[1], X. Li[1], Y. Aoki[1], M. Hiratsuka[1], T. Suormala[2], R.E. Baumgartner[2], K.M. Gibson[3], K. Narisawa[1].* 1) Dept of Medical Genetics, Tohoku Univ School of Medicine, Sendai, Japan; 2) Metabolic Unit, University Children's Hospital, Basel, Switzerland; 3) Biochemical Genetics Laboratory, Dept of Molecular and Medical Genetics, Oregon Health Sciences University, Portland, OR.

Holocarboxylase synthetase (HCS) deficiency is a metabolic disorder that causes a biotin-responsive multiple carboxylase deficiency. We analyzed the kinetic properties of seven mutant HCS proteins. Two of these enzymes harbored mutations within the putative biotin-binding region of HCS and showed elevated Km values for biotin compared to that of the wild-type form (Km mutant; Gly581Ser: 45 times, delThr610: 3 times). The remaining five mutations (Arg183Pro, Leu216Arg, Leu237Pro, Val333Glu, and Val363Asp) were located outside the biotin-binding region. The enzymes containing these mutations showed normal or low Km values for biotin (non-Km mutant). Symptoms of patients who have the non-Km mutants, as well as those of patients who have the Km mutants, responded to biotin therapy. This is probably because the Km value for biotin of normal HCS is higher than the physiological concentration of biotin in human cells. The Vmax values of all mutant HCS proteins were considerably decreased, but to a variable degree. The responsiveness to biotin supplementation of propionyl-CoA carboxylase activity in cultured cells bearing the mutations correlated well with the degree of reduction in the Vmax of HCS. Patients who have mutant HCS proteins with lower Vmax showed poorer clinical and biochemical responses to biotin therapy. These observations suggest that the reduction of Vmax is an essential factor for pathophysiology and prognosis of HCS deficiency under treatment with large amounts of biotin. The determination of HCS genotype can be valuable for characterizing the clinical phenotype in HCS deficient patients.

Program Nr: 2440 from the 1999 ASHG Annual Meeting

Novel mutations of the Glutaryl-CoA Dehydrogenase Gene in Three Chinese patients with Glutaric aciduria Type I. *N.L.S. Tang[1], L.K. Law[1], J. Hui[2], K.L. Cheung[2], Y.Y. Lam[3], W.L. Yeung[2], T.F. Fok[2].* 1) Department of Chemical Pathology, The Chinese University of Hong Kong, Hong Kong SAR, China; 2) Department of Paediatrics, The Chinese University of Hong Kong, Hong Kong SAR, China; 3) Department of Paediatrics, Kwong Wah Hospital, Hong Kong.

Glutaric aciduria Type I (GA-I) is an autosomal recessive disorder caused by inheritance of defective glutaryl-CoA dehydrogenase (GCDH). The enzyme converts glutaryl-CoA to crotonyl-CoA in the breakdown of lysine, hydroxylysine and tryptophan. GCDH deficiency led to urine excretion of glutaric acid. Patients present with progressive neurologic mansifestations or acute encephalopathic crisis. Mutations in GCDH gene were responsible for GA-I. Genetic heterogeneity was reported and more than 60 mutations had been reported. We reported here the GCDH mutations in three Chinese patients with GA-I. All patients excreted significant amount of glutaric acid in urine and were picked up by urine organic acid study. Two of them had also been confirmed by fibroblast enzyme assay. The 11 exons were amplified by intron-based primers and examined by SSCP and then sequencing of SSCP variants. Five mutations were found and four of them were novel. R355H was a previously reported mutation. Three mutations were missense including; A219T, A298T, R386G. One patient who is the second child of a consanguineous marriage is homozygote for a splicing mutation, IVS10-2A->C. This mutation is predicted to results in a GCDH without exon 11. We did not find a common mutation in the Chinese population. The absence of a common mutation is also a feature in other ethnic groups. All mutations were found downstream of exon 6. It may be related to the hypothesis that the carboxyl end of the enzyme contains important catalytic sites.

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Program Nr: 2441 from the 1999 ASHG Annual Meeting

Linkage Analysis Of A Family With Autosomal Recessive Vitamin B12 Deficiency. *M.K. Tayeh[1], J. Al-Alami[1], M.Y. Al-Sheyyab[1], S.M. Leal[2], H.E. El-Shanti[1].* 1) Jordan University of Science & Technology, Irbid, Jordan; 2) The Rockefeller University, New York, NY, USA.

Inherited disorders of vitamin B12 metabolism are known as single gene defects, mostly transmitted in an autosomal recessive mode. They affect absorption, transport, or intracellular metabolism of vitamin B12. Patients with vitamin B12 absorption and transport defects demonstrate at least megaloblastic anemia as a characteristic change besides other manifestations. We identified a large inbred kindred with congenital megaloblastic anemia from Northern Jordan. Linkage analysis was undertaken to map the locus responsible for the disease in this family. The first attempt was at excluding linkage to three known loci responsible for a similar clinical picture. These loci are the MGA1 locus causing Imerslund-Grasbeck syndrome (IGS), the intrinsic factor causing congenital pernicious anemia (CPA), and TCII causing transcobalamin II deficiency. Exclusion of linkage to these three candidate loci on chromosome 10, 11, and 22 was indicated by absence of homozygosity in the affected individuals and the significant LOD scores (less than -2 at different recombination fractions) for all the used markers. It is then highly suggested that the disorder in this family is not due to IF, MGA1 or TCII gene defects. This finding either demonstrates that CPA or IGS is genetically heterogeneous with more than the two expected loci involved or it indicates that there might be another unidentified step involved in the absorption or transport of vitamin B12.

Program Nr: 2442 from the 1999 ASHG Annual Meeting

Molecular prenatal diagnosis of carnitine palmitoyltransferase 2 deficiency. *L. Thuillier[1], H. Belbachir[1], G. Royer-Legrain[2], T. Attie-Bitach[2], A. Driben[2], N. Abadi[1], P. Kamoun[1], J.M. Saudubray[3], A. Munnich[2], J.P. Bonnefont[1, 2]*. 1) Genetic Biochemistry Unit; 2) Department of Genetics; 3) Department of Pediatrics, Hopital Necker-Enfants Malades, Paris, France.

Carnitine palmitoyltransferase 2 (CPT2) deficiency is one of the most common inherited disorders of mitochondrial long-chain fatty acid oxidation. This disease has two clinical presentations: the “muscle“ phenotype is a benign condition affecting teenagers and young adults, while the “hepatocardiomuscular“ form exposes to a high risk of sudden death in neonates or infants. We were asked for genetic counseling in a couple of first cousins whose first child was affected with CPT2 deficiency. Clinical features included hypoketotic hypoglycemia, hepatomegaly, cardiomyopathy with heart beat disorders, and tubulopathy at one day of life. After initial recovery, the child suddenly died at 22 months of age. CPT2 activity was less than 5% of control values in fibroblasts. Analysis of the full-length coding sequence of the CPT2 gene by DGGE-sequencing revealed a homozygous A®G transition at nt 1500, predicting an Asp®Gly substitution. This substitution of an aminoacid highly conserved across species and CPT isoforms was not found in 50 control DNAs, thus suggesting it was the disease-causing mutation. During next pregnancy, genomic DNA from a chorion villus sampling was submitted to both D328G detection and indirect study using polymorphic markers linked to the CPT2 locus at 1p32. Both approaches established that the fetus had inherited the mutant CPT2 allele in a heterozygous state, and was therefore CPT2 deficient. Parents chose not to terminate the pregnancy, and delivery should occur within next months. This is to our knowledge the first report establishing that DNA analysis is a convenient approach to an early prenatal diagnosis of CPT2 deficiency.

Program Nr: 2443 from the 1999 ASHG Annual Meeting

Mutation of the thermolabile variant of MTHFR enzyme in a family with vascular events and neural tube defects. *G. Toth[1], E. Morava[1], K.E. Jackson[2], A. Horváth[3], M. Czakó[1], Gy. Kosztolányi[1]*. 1) Department of Medical Genetics & Child Development, POTE, Pecs, Hungary; 2) Human Genetics Program, Tulane University School of Medicine, New Orleans, LA; 3) Department of Gynecology, POTE, Pecs, Hungary.

Homozygous mutation of the thermolabile variant of methylene tetrahydrofolate reductase (MTHFR) may result in hyperhomocystinemia leading to an increased risk for cardiovascular accidents and neural tube defects. According to recent studies heterozygosity for MTHFR enzyme mutation is also more frequent in patients with thrombotic disease compared to that in the average population. We report a family with different types of early vascular disease in three consecutive generations. MTHFR heterozygosity was detected in the proband and in her mother and grandfather. The patient's aunt died of spina bifida shortly after birth. Our patient's sister with spina bifida occulta also carries the same mutation, as does her daughter who is asymptomatic. In the other asymptomatic members of the family no mutations were found. Unexpectedly, hyperhomocystinemia was detected in the heterozygote individuals. On continuous folate supplementation of 1 mg/day, no homocystinemia is detectable. Our study demonstrates the necessity for folic acid therapy in mutation carriers to prevent early vascular events, and also to reduce the risk of neural tube defects in a preconception setting.

Program Nr: 2444 from the 1999 ASHG Annual Meeting

Xenomitochondrial mouse cell cybrid models of multiple respiratory chain complex deficiency. *I. Trounce, M. McKenzie.* Mutation Research Centre, Melbourne, Victoria, Australia.

The lack of mouse cell mtDNA mutants presents a barrier to producing cell culture and mouse models of mitochondrial DNA disease. We have used a similar approach to that recently reported for primate/human cybrids (Kenyon & Moraes, PNAS,94:9131,1997), to produce xenomitochondrial mouse cybrids. Fusion of enucleated hamster cells (Mus/hamster divergence was around 16 m.y.b.p.) with *Mus domesticus* rho-zero cells followed by selection for respiratory competence failed to produce any cybrids. Similar crosses with *Rattus norvegicus* cells (*Mus/Rattus* divergence was around 10 m.y.b.p.) and *Mus spretus* cells (divergence around 1 m.y.b.p.) produced cybrids at a high frequency (around one cybrid per 1,000 cells). *Rattus* cybrids grew more slowly and acidified media more quickly than *M. spretus* cybrids or *M. domesticus* reconstituted cells. Polarographic analysis revealed a severe respiratory deficiency of NADH-linked and FAD-linked substrate oxidation in the *Rattus* cybrids compared with preserved function in the *M. spretus* and control cybrids. Spectrophotometric analysis of individual respiratory chain complexes in the cybrids showed the *Rattus* respiratory defect to result from partial deficiencies of complexes I (60% control), III (20%) and IV (60%). The *M. spretus* cybrid showed partial defects of complexes I (60%) and IV (60%) which did not impact on overall flux as measured with the polarograph. Metabolic labeling of mitochondrial translation products using labelled methionine in the presence of cyclohexamide showed normal or increased production of mtDNA-encoded proteins in the *Rattus* cybrids. Using oxidation of the peroxide-sensitive probe DCFDA-H2 as a measure of hydrogen peroxide production, we found that the amount of peroxide produced per unit of oxygen consumed was increased in the *Rattus* and *M. spretus* cybrids. We conclude that the *Rattus* xenocybrids exhibit a model of multiple respiratory chain dysfunction as seen in some severe human mitochondrial DNA diseases. The defects in this model can be used to investigate nuclear-mitochondrial subunit interactions in respiratory complex assembly and function.

Program Nr: 2445 from the 1999 ASHG Annual Meeting

De novo purine biosynthesis enzyme interactions: yeast two hybrid studies. *G.N. Vacano, D. Patterson.* Patterson Lab, Eleanor Roosevelt Inst, Denver, CO.

Purines are fundamental biological molecules that serve a number of roles, including components of nucleic acids, neurotransmitters, vasodilators, second messengers, coenzymes, and key molecules in cellular energy metabolism. Errors in purine metabolism can result in mental retardation, autism, sensorineural deafness, and arthritis. The major source of purines, the de novo pathway, has not been well characterized at the molecular level. There is evidence, including co-sedimentation through sucrose gradients and co-purification gel chromatography, which suggests that the enzymes in the de novo pathway may form multi-enzyme complexes. These experiments have not conclusively demonstrated the existence of a classical enzyme complex, because of the weakness of the interactions. To investigate the nature of protein interactions involving the de novo pathway enzymes, bait and prey vectors containing entire or partial cDNA sequences were constructed, and these were used in yeast two hybrid studies to identify and characterize discrete interactions.

Program Nr: 2446 from the 1999 ASHG Annual Meeting

A MITOCHONDRIAL CYTOCHROME b MUTATION BUT NO MUTATIONS OF NUCLEARLY ENCODED SUBUNITS IN UBIQUINOL CYTOCHROME c REDUCTASE (COMPLEX III) DEFICIENCY. *I. Valnot, D. Chretien, P. de Lonlay, A. Munnich, P. Rustin, A. Rotig.* INSERM U393, Hosp Necker, Paris, France.

Ubiquinol cytochrome c reductase (complex III) deficiency represents a clinically heterogeneous group of mitochondrial respiratory chain disorders which can theoretically be subject to either a nuclear or a mitochondrial mode of inheritance. In an attempt to elucidate the molecular bases of the disease, we first determined the nucleotide sequence of three unknown subunits (9.5 kDa, 7.2 kDa, 6.4 kDa) by cyberscreening of human EST data bases and we sequenced the 11 cDNA subunits encoding complex III in five patients with isolated complex III deficiency. No mutation in the nuclearly encoded complex III subunits was observed but a mutation in the cd2 helix of the mitochondrial (mt) cytochrome b gene was found to alter the conformation of the bc1 complex in one patient with severe hypertrophic cardiomyopathy. The present study is highly relevant to genetic counseling as absence of mtDNA mutations in all but one patient in our series strongly supports autosomal rather than maternal inheritance in the majority of patients with complex III deficiency.

Program Nr: 2447 from the 1999 ASHG Annual Meeting

Biochemical, genetic and immunoblot analyses of 17 patients with an isolated cytochrome c oxidase deficiency. *J. Von Kleist-Retzow[1], E. Vial[1], K. Chantrel-Groussard[1], A. Rotig[1], A. Munnic[1], P. Rustin[1], J.W. Taanman[2].* 1) INSERM U393, Hosp Necker, Paris, France; 2) Royal Free and University College Medical School, Dep of Clinical Neurosciences, Rowland Hill Street, London NW3 2PF, UK.

Mitochondrial respiratory chain defects involving cytochrome c oxidase (COX) are found in a clinically heterogeneous group of diseases yet the molecular basis of these disorders have been determined in only a limited number of cases. Here, we report the clinical, biochemical and molecular findings in 17 patients who all had isolated COX deficiency and expressed the defect in cultured skin fibroblasts. Immunoblot analysis of mitochondrial fractions with nine subunit specific monoclonal antibodies revealed that in most patients, including in a patient with a novel mutation in the SURF1 gene, steady-state levels of all investigated COX subunits were decreased. Distinct subunit expression patterns were found, however, in different patients. The severity of the enzymatic defect matched the decrease in immunoreactive material in these patients, suggesting that the remnant enzyme activity reflects the amount of remaining holo-enzyme. Four patients presented with a clear defect of COX activity but had near normal levels of COX subunits. An increased affinity for cytochrome c was observed in one of these patients. Our findings indicate a genetic heterogeneity of COX deficiencies and are suggestive of a prominent involvement of nuclear genes acting on the assembly and maintenance of cytochrome c oxidase.

Program Nr: 2448 from the 1999 ASHG Annual Meeting

Subdural hematomas and retinal hemmorhages: Glutaric Aciduria type 1 or Shaken Baby Syndrome? *I.R. WALSH, G.W. HORNIG, A.H. GETTEL, M.J. DASOUKI.* PEDIATRICS, CHILDREN MERCY HOSPITAL, KANSAS CITY, MO.

A 10 month old boy presented with increasing head circumference, irritability, ataxia and frequent falls. A head CT scan showed bilateral subdural hematomas while a previuos study a week earlier was normal. Physical examination was significant for macrocephaly, hypotnia and retinal hemorrhages. Urine organic acid and plasma acyl carnitine profiles showed extremely elevated glutaric, 3-hydroxyglutaric acids and C5-dicarboxylic acylcarnitine while free and total carnitines were extremely low. A skeletal survey was normal. Normal CPK, mild hepatic dysfunction and mild hyperammonemia were also seen. At 14 months of age, his head MRI showed abnormal brain myelination, global atrophy espcially in the frontotemporal region and signal abnormality within the basal ganglia and periventricular white matter and centrum semiovale. Despite prompt evacuation of his subdural hematomas, early institution of restricted protein diet, carnitine and riboflavin supplementation, he progressed to develop seizures, severe irritability and dystonia. Initially, Lorazepam, Carbidopa/Levodopa, clonidine and midazolam had little effect on his irritability and dystonia. Multiple Botulinum toxin (Botox) intramuscular injections produced the best response for the dystonia. Glutraic aciduria type 1 is an autosomal recessive inborn error of metabolism of tryptophan, lysine and hydroxylysine that results from glutaryl CoA dehydrogenase (GDD) deficiency. Clinical presentation is variable ranging from no symptoms to acute encephalopathic metabolic crisis. Among a variety of radiologic findings including frontotemporal atrophy and basal ganglia changes are most characteristic. While subdural effusions are not uncommon in GA1, isolated subdural hematomas were reported in a few patiens with GA1 and only in 1 patient had both retinal hemorrhages and subdural hematoma. The absence of subdural effusion in our patient demonstrates that an effusion is not requried for the hematoma formation. Also, the lack of signs of non-accidental trauma suggests that the combination of subdural hematomas and retinal hemorrhages is etiologically related to GA1.

Program Nr: 2449 from the 1999 ASHG Annual Meeting

Hormone-Sensitive Lipase (HSL) is Not Essential for Adipose Tissue Lipolysis. *S.P. Wang[1], N. Laurin[1], L. Pan[1], M.F. Robert[1], M. Ashmarina[1], L. Oligny[1], J.M. Trasler[2], M.A. Rudnicki[3], L. Hermo[2], E. Levy[1], G.A. Mitchell[1]*. 1) Serv Genet Med and Gastroent (EL) and Dept Pathol (LO), Hop Sainte-Justine, Montreal, Canada; 2) Montreal Children's Hosp (JT) and Dept Anat (LH), McGill U, Montreal; 3) Inst Molec Biol & Biotech, McMaster U, Hamilton.

HSL is felt to be necessary for fat release from adipose tissue. HSL can hydrolyse a variety of fatty acyl esters other than triglycerides, including cholesteryl esters. It is also expressed in many nonadipose sites including pancreatic beta cells, macrophages and steroid-synthesizing tissues like adrenals and gonads. To directly assess the biological importance of HSL, we performed constitutive gene targeting at the HSL locus in mice. Unexpectedly, HSL-deficient mutant (mut) mice had normal body weight, abdominal fat mass and adipose tissue histology. Adipocytes isolated from perigonadal fat of HSL-deficient mice were small (cell diameter 70 ± 3.7 mm vs 77 ± 5.7 in heterozygotes (het) and 81 ± 7.6 in wild type (wt) mice) and basal lipolysis was increased (4.45 ± 0.85 nmol glycerol/10^8 mm^2/h vs 3.35 ± 1.40 in hets and 2.75 ± 1.05 in wt mice; $p<0.03$, mut vs wt). As expected, however, adrenergic stimulation of lipolysis by the beta-3 agonist CL316,243 was undetectable for mutant mice (1.08 ± 0.12-fold vs 4.31 ± 1.26 for hets and 6.49 ± 1.65 for wt; $p<0.01$, mut vs wt). We are currently investigating the function of other organs in which HSL is expressed. Our results suggest a previously-unsuspected redundency in adipocyte lipolysis, in which HSL mediates hormone-responsive lipolysis and an HSL-independent pathway mediates basal lipolysis. HSL may be important in fasting and the fight-or-flight response, in which lipolysis is activated in a hormone-dependent fashion. The delineation of the HSL-independent pathway and of its interaction with HSL may be relevant to obesity.

Program Nr: 2450 from the 1999 ASHG Annual Meeting

Missense mutations in the organic cation transporter OCTN2 in patients with primary carnitine deficiency. *Y. Wang[1], F. Taroni[2], B. Garavaglia[2], T. Cowan[3], J. Ye[1], N. Longo[1]*. 1) Medical Genetics/Pediatrics, Emory University, Atlanta, GA; 2) Istituto Nazionale Neurologico C.Besta, Milano, Italy; 3) University of Maryland, Baltimore, MD.

Primary carnitine deficiency (OMIM 212 140) is an autosomal recessive disorder of fatty acid oxidation caused by defective carnitine transport. This disease presents early in life with hypoketotic hypoglycemia or later in life with skeletal myopathy or cardiomyopathy. The transporter defective in primary carnitine deficiency is named Organic Cation Transporter with Nucleoside binding site-2 (OCTN2) and mutations abolishing its function were recently demonstrated in a few patients. Here we extend mutational analysis of OCTN2 to additional families with primary carnitine deficiency. Fibroblasts obtained from different patients expressed the OCTN2 gene and had either completely absent or impaired (2-4% of control) saturable carnitine transport. Fibroblasts from two patients with absent carnitine transport were homozygous or compound heterozygous for mutations producing premature STOP codons (R282X, Y401X, and 458X). Fibroblasts from four patients retained residual carnitine transport and carried missense mutations (R169W, G242V, W351R, E452K). Two mutations (G242V and W351R) affected transmembrane domains, while two others (R169W and E452K) were in intracellular loops. The R169W mutation was found in two unrelated families and occurred on two different RFLP haplotypes in an apparently homozygous patient. Expression studies in CHO cells indicated that the E452K-mutant OCTN2 cDNA impaired carnitine transport to levels corresponding to 3% of those measured with the normal OCTN2 cDNA, correlating well with the transport data in homozygous human fibroblasts. As expected, no increase in carnitine transport was observed in CHO cells expressing nonsense mutations in the OCTN2 cDNA. These results indicate that nonsense mutations in OCTN2 abolish saturable carnitine transport, while some missense mutations retain residual carnitine transport. The incomplete impairment of transporter function caused by missense mutations may be important in determining the severity of phenotypic expression.

Program Nr: 2451 from the 1999 ASHG Annual Meeting

Cellular and Molecular Studies of Smith-Lemli-Opitz Syndrome. *C.A. Wassif[1], P.A. Krakowiak[1], N. Nwokoro[1], M. Tsokos[3], W.E. Connor[2], C.M. Maslen[2], R.D. Steiner[2], F.D. Porter[1]*. 1) HBD, NICHD/NIH, Bethesda, MD; 2) Oregon Health Sciences University, Portland, OR; 3) NCI, NIH, Bethesda, MD.

The Smith-Lemli-Opitz Syndrome (SLOS) is an autosomal recessive malformation syndrome due to mutations in the 7-dehydrocholesterol reductase gene. In the last step of endogenous cholesterol biosynthesis 7-dehydrocholesterol reductase catalyzes the conversion of 7-dehydrocholesterol to cholesterol. Individuals with SLOS have elevated levels of 7-dehydrocholesterol. The molecular, biochemical and cellular mechanisms by which this inborn error of metabolism cause the SLOS phenotype have not been determined. In order to establish a genotype/phenotype correlation for this malformation syndrome we have cloned the human 7-dehydrocholesterol reductase gene and identified multiple mutations in patients with SLOS. The most common mutation is G to C transversion at the -1 position of a splice acceptor. When homozygous, this mutation gives rise to a severe phenotype. All of the mutations result in elevated levels of 7-dehydrocholesterol. These abnormal levels of 7-DHC appear to perturb the function of proteins that contain sterol sensing domains. The sonic hedgehog receptor Patched and the Neiman Pick type C (NPC) gene both encode sterol sensing domains. Previously it has been shown that inhibitors of cholesterol biosynthesis impair the signaling of sonic hedgehog through Patched. SLOS has phenotypic overlap with sonic hedgehog mutations. Thus, abnormal sterol biosynthesis in SLOS may perturb Patched signaling during development and thereby may explain the genesis of some of the developmental malformations. We now demonstrate that SLOS fibroblasts have a secondary defect in intracellular LDL cholesterol metabolism. Like NPC cells, SLOS fibroblasts show increased filipin staining, and can develop lysosomal inclusion bodies similar to those seen in NPC. In addition, SLOS fibroblasts have impaired LDL degradation. We propose that these findings are due to inhibition of NPC1 function through its sterol sensing domains. Clinically, this secondary defect in LDL cholesterol transport may limit the effectiveness of the current dietary therapy.

Program Nr: 2452 from the 1999 ASHG Annual Meeting

Analysis of the N-Acetyl-a-D-glucosaminidase gene in four patients with Sanfilippo syndrome type B: Identification of three novel mutations and correlation of enzymatic activity with genotype in two families. *T.C. Wood, J.N. Thompson.* Dept Human Genetics, Univ Alabama at Birmingham, Birmingham, AL.

Mucopolysaccharidosis IIIB (Sanfilippo Syndrome Type B, MIM 252920) is caused by a deficiency in the lysosomal enzyme N-acetyl-a-D-glucosaminidase (NAG). This enzyme is involved in the breakdown of heparan sulfate and a deficiency in the enzyme causes the lysosomal storage of partially degraded substrate. Over time this storage disrupts normal cellular function causing the disease phenotype. MPS IIIB individuals are characterized by mild somatic features and degenerative neurological problems that can include a wide spectrum of behavioral disturbances. The NAG gene has been localized to chromosome 17q21.1 and the complete sequence of the gene has been reported. In the present study, we report the identification of 3 previously described mutations (R297X, R643H, Y140C) and 3 novel mutations (W103R, S500I, P604L) from 4 patients enzymatically diagnosed with MPS IIIB. Each novel mutation occurs at an amino acid that is conserved between human and mouse. Family analysis from a R297X homozygote showed the brother of the proband to be a carrier of the R297X mutation. Using cultured fibroblast homogenates NAG activity was not detected in the homozygous individual while his brother was found to have only 20% of the activity of the control. In addition, analysis of a family in which the proband was a compound heterozygote for the Y140C and W103R mutations showed the father, brother, and sister to be carriers of the W103R mutation. The mother and maternal aunt of the proband both contained the Y140C mutation. Serum NAG activity for each carrier was found to be only 24-36% of control values while the affected individual had no detectable serum activity. These findings support the observations of Vance et al. (1981 Clinical Genetics 20:135-140) that serum NAG activity is significantly decreased in heterozygous individuals.

Program Nr: 2453 from the 1999 ASHG Annual Meeting

Further characterization of Wilson disease in Taiwan: identification of two novel mutations and high correlation between haplotype and mutation. *J.Y. Wu[1], F.J. Tsai[2], C.C. Lee[3], J.G. Chang[1], C.H. Tsai[2].* 1) Dept Medical Research, China Medical Col Hosp, Taichung, Taiwan; 2) Dept Pediatrics, China Medical Col Hosp, Taichung, Taiwan; 3) Dept Neurology, China Medical Col Hosp, Taichung, Taiwan.

Wilson disease is caused by the deficiency of copper-transporting enzyme, P-type ATPase (ATP7B). Twelve different mutations had previously been identified in Taiwanese Wilson disease chromosomes. We report here the finding of four newly identified missense mutations in Taiwanese Wilson disease chromosomes, two of which are novel. We also did haplotype analysis of Taiwanese WND chromosomes by the use of three well-characterized STR markers (haplotype was assigned in the order of D13S314-D13S301-D13S316). We found association between some mutations and their respective haplotypes. R778L was found to be associated with haplotypes 8-4-4 and 8-4-5.5. P992L was found to be associated with haplotypes 8.5-6.5-2 and 8.5-6.5-6.5. R778Q and G943D were found to be exclusively associated with haplotype 7.5-0.5-5.5 and haplotype 11-1.5-5.5, respectively. The haplotype deduced pedigree analysis was shown to be helpful in mutation analysis of WND chromosomes and in molecular assessment of either pre-symptomatic WND patients or carriers. Given the complexity and heterogeneity of mutation spectrum of ATP7B, haplotype data is important in assisting mutation analysis and carrier detection of Wilson disease.

Program Nr: 2454 from the 1999 ASHG Annual Meeting

Molecular characterisation of two mutations that cause severe and intermediate Sanfilippo syndrome type B. *G. Yogalingam, B. Weber, J.J. Hopwood.* Lysosomal Diseases Research Unit, Department of Chemical Pathology, Women's and Children's Hospital, North Adelaide, SA, 5006, Australia.

To investigate the relationship between genotype, cellular biochemistry and clinical phenotype in A-N-acetylglucosaminidase (NAG) deficient mucopolysaccharidoses type IIIB (MPS IIIB) patients, two mutations, F48L and R297X, which are associated with intermediate and severe Sanfilippo phenotypes respectively, were engineered into the wild-type NAG cDNA. The R297X allele displayed no detectable NAG activity when expressed in Chinese hamster ovary (CHO-KI) cells (CHOR297X) and was observed only as a 34 kDa truncated polypeptide that was rapidly degraded. Skin fibroblasts obtained from a R297X/R297X homozygous patient contained no detectable NAG activity and a twelve-fold elevated level of ^{35}S-labelled glycosaminoglycan (GAG) storage when compared with normal fibroblasts. The lack of detectable NAG activity in CHOR297X cells and R297X homozygous skin fibroblasts and the highly elevated levels of ^{35}S-labelled GAG storage in R297X homozygous skin fibroblasts are consistent with the clinically severe phenotype associated with the R297X allele. When over-expressed in CHO-KI cells, F48L NAG was detected as a 89 kDa precursor polypeptide that was not cleaved to the mature 79 kDa form or secreted into the medium. When expressed via retroviral-mediated gene transfer in R297X homozygous MPS IIIB skin fibroblasts, intracellular F48L NAG activity corresponded to 3.75 % of NAG activity levels in wild-type NAG-transduced MPS IIIB fibroblasts. This residual level of NAG activity was sufficient to metabolise 34 % of intracellular ^{35}S-labelled GAG storage suggesting that some F48L NAG is being correctly sorted to the lysosomal compartment. The residual level of F48L NAG activity in the lysosomes of F48L NAG-transduced MPS IIIB fibroblasts and subsequent partial turn-over of GAG is consistent with the intermediate clinical phenotype associated with the F48L allele.

Program Nr: 2455 from the 1999 ASHG Annual Meeting

Two Korean patients with citrullinemia presenting with a stroke-like episode. *H. Yoo[1], H. Kim[2]*. 1) Dept Pediatrics, Asan Medical Ctr, Seoul, Republic of Korea; 2) Dept Pediatrics, Ulsan Univ Hosp, Ulsan, Republic of Korea.

Urea cycle disorders are characterized by encephalopathy, respiratory alkalosis and hyperammonemia. A urea cycle disorder should be considered as a diagnosis in a patient with occult encephalopathy regardless of age. The most common central nervous system pathology is cerebral edema. The cerebral edema is caused by astrocyte swelling secondary to hyperammonemia and intracellular glutamine accumulation. Strokes in children occur in conjunction with cardiac disease, hematologic disorders, mitochondrial encephalopathy, trauma, intracranial infection, and rarely inborn errors of metabolism. To our knowledge, there have been several reports on patients with ornithine transcarbamylase deficiency presenting with strokes. However, a case of citrullinemia presenting with a stroke-like episode has not been described previously. We report two unrelated Korean infants with citrullinemia with an initial clinical manifestation of stroke, emphasizing their clinical features, brain MR imaging and their genotypes. The differential diagnosis for unexplained strokes should include inborn errors of urea cycle metabolism.

Program Nr: 2456 from the 1999 ASHG Annual Meeting

Cytochrome P450 2A6 (CYP2A6): a population genetics study using a new genotyping approach. *C.P. Zabetian[1,2], J. Gelernter[1,2], H.R. Kranzler[3], J.F. Cubells[1,2].* 1) Dept. of Psychiatry, Yale University, New Haven, CT; 2) Dept. of Psychiatry, VA CT Healthcare System, West Haven, CT; 3) Dept. of Psychiatry, U. Conn. Health Ctr., Farmington, CT.

CYP2A6 is the predominant enzyme responsible for the metabolism of nicotine to cotinine. Several alleles at the CYP2A6 locus have been identified including CYP2A6*1, which encodes an active enzyme, and two null alleles (CYP2A6*2 and *3) encoding inactive proteins. It has been proposed that the presence of one or more null alleles may protect against tobacco-dependence (Pianezza et al., Nature 393: 750, 1998). However, different laboratories have reported widely disparate CYP2A6 allele frequencies in Caucasians. This could be due to either actual allele frequency differences, or to methodological differences. We therefore developed an improved method to genotype CYP2A6*1, *2 and *3, and assessed allele frequencies in populations of diverse geographic origin.

Avoiding coamplification of two genes (CYP2A7, CYP2A13) and two pseudogenes that bear >90% sequence homology to CYP2A6 is critical in obtaining accurate results. Using a combination of novel and previously described primers, nested PCR, and differential restriction enzyme digestion, we developed a method that offers the following advantages. 1) All primers are anchored in exonic regions, decreasing the likelihood of allele dropout from primer mismatches in more polymorphic untranslated areas. 2) The first PCR product is shorter, and thus technically easier to amplify. 3) Both of the null alleles discussed above are detected.

We ascertained CYP2A6 genotypes in samples from European-Americans (EAs), African- Americans, Japanese, Ashkenazi and Ethiopian Jews, and Bedouins. The combined null allele frequency in EAs was 2-3%, and did not exceed 6% in the remaining 5 populations. Although these results confirm the presence of population variation in CYP2A6 allele frequencies, they suggest the null alleles CYP2A6*2 and *3 are less common than previously reported by most authors. Further study of the population genetics of CYP2A6 is critical in evaluating the role of this gene in nicotine addiction.

Program Nr: 2457 from the 1999 ASHG Annual Meeting

Refined localization of hKCA3/KCNN3 gene by haplotype analysis and allele sizing to 1q21, between D1S506 and the Duffy gene. *S. Hanz[1,2], N. Parasol[1], N.S. Kosower[1], R. Navon[1,2].* 1) Dept Human Genetics, Tel-Aviv Univ, Tel-Aviv, Israel; 2) Molecular Genetics, Meir Hospital, Sapir Medical Center, Kfar Sava, Israel.

The chromosomal localization of hKCA3/KCNN3, a neuronal small conductance calcium-activated potassium channel gene, was recently mapped by us to 1q21 using FISH analysis. It was recently reported that the hKCA3/KCNN3 gene might be involved in schizophrenia. The gene contains two adjacent polyglutamine repeats arrays in its N-terminal region. The second repeat is highly polymorphic in Caucasian European and North American populations. In a previous work we studied the involvement of 1q in schizophrenia. To establish haplotypes of members from schizophrenic families, we used 15 markers from 1cen-1q23, most of them highly polymorphic microsatellite CA repeats probes. Haplotype analysis and allele sizing of the polymorphic CAG repeats on 84 members of 8 non-Ashkenazi Jewish families enabled us to refine the localization of hKCA3/KCNN3 gene. The localization was mainly based on one family, containing multiple cross-overs. Combination of the two methods, haplotype analysis and allele sizing, enabled us to refine the localization of the hKCA3/KCNN3 gene to the region between the marker D1S506 and the Duffy gene. The localization was consistent with the haplotypes of other informative families tested.

Program Nr: 2458 from the 1999 ASHG Annual Meeting

Chromosome 14 linkage analysis for atopic asthma in Italian families, and mutation study of two serpin genes. *G. Malerba[1], C. Patuzzo[1], E. Trabetti[1], C. Lauciello[1], R. Galavotti[1], M. Wahlen[1], T. Scherpbier[2], R. Levitt[2], L. Pescollderungg[3], G. Zanoni[4], A.L. Boner[1], P.F. Pignatti[1].* 1) Mother-Child and Biol-Genet, University of Verona, Italy; 2) Magainin Inst. Mol. Med., Plymouth Meeting, PA; 3) Istitute of Paediatrics, Hospital of Bolzano, Italy; 4) Dept. of Pathology, University of Verona, Italy.

In order to identify genetic factors for susceptibility to atopy and asthma in childhood we have collected 116 families (560 subjects) with at least one affected sibpairs, from a restricted geographic area in North East Italy. All the subjects were characterized for the following phenotypes: atopy, total serum IgE, Skin Prick Test (SPT), Bronchial Hyperresponsiveness (BHR) to methacoline and clinical asthma. Atopy was defined as increased IgE and/or positive SPT. A chromosome 14 region of 118 cM has been studied with 14 DNA markers (D14S742, D14S1280, D14S608, D14S306, D14S583, D14S587, D14S588, D14S606, D14S616, D14S617, D14S1434, D14S605, D14S614, D14S1426). Multipoint analysis indicated linkage of BHR with D14S617 (NPL=2.22, p=0.013). TDT analysis displayed a significant disequilibrium of D14S617 alleles and BHR (p=0.02). The region in which D14S617 is located (14q32) is different from that reported to be linked to asthma in 14q11.2-13 (CSGA, Nat Genet. 1997; 15:389). Alpha 1 antitrypsin (AT) and alpha 1 antichymotrypsin (ACT) genes map at 1 Mb from D14S617. These serine protease inhibitors have been reported to be associated with chronic obstructive pulmonary disease. We have analyzed Z, S, and the Taq I mutations in the AT gene and the Thr-15Ala signal peptide polymorphism in the ACT gene. Linkage of any of the 4 mutations with any phenotype did not show any significant result. A transmission disequilibrium was observed between the Thr-15Ala mutation and total IgE (p=0.014), asthma (p=0.027) or BHR (p=0.05). In conclusion, the alpha 1 antichymotrypsin gene or a closely located gene may be involved in susceptibility to atopic asthma in this group of patients.

Program Nr: 2459 from the 1999 ASHG Annual Meeting

Mapping of a new autosomal dominant non-syndromic hearing loss to chromosome 15q26. *M. Mangino[1], F. Sangiuolo[1], F. Capon[1], G. Novelli[1], E. Carraro[2], F. Gualandi[3], M. Mazzoli[2], A. Martini[2], B. Dallapiccola[1,4]*. 1) Department of Biopathology, Tor Vergata University of Rome and CSS Mendel Institute, Rome; 2) ENT Department, University of Ferrara, Italy; 3) Department of Genetics, University of Ferrara, Italy; 4) IRCCS, CSS, S.Giovanni Rotondo, Italy.

Hearing loss is the most frequent sensory defect characterized by wide heterogeneity. We analyzed a four-generation Italian family in which a non-syndromic hearing impairment is transmitted as an autosomal dominant trait. Patients showed a sensorineural hearing impairment involving middle and high frequencies, moderate to severe grade. The hearing impairment starts at the end of the first decade, rapidly progressive till the fourth decade, then it becomes stable. After excluding known DFNA loci using markers listed on the Hereditary Hearing Loss Homepage (URL: http://dnalab-www.uia.ac.be/dnalab/hhh), we performed a genome wide scan with 358 highly informative microsatellite markers using the ABI PRISM Linkage Mapping Set (PE Applied Biosistem,USA). Significant linkage (Zmax=3.37;q=0) was obtained with markers of chromosome 15q26. Haplotype analysis from genotype of affected and unaffected individuals defined a 20cM disease-gene interval. Fine mapping and identification of candidate genes are in progress.

Program Nr: 2460 from the 1999 ASHG Annual Meeting

The gene for Cherubism maps to chromosome 4p16.3. *J. Mangion[1], N. Rahman[1], S. Edkins[1], R. Barfoot[1], T. Nguyen[1], A. Sigurdsson[1], J.V. Townend[2], D.R. Fitzpatrick[3], A.M. Flannagan[4], M.R. Stratton[1]*. 1) Cancer Genetics, Institute of Cancer Research, Sutton, Surrey, UK; 2) Department of Maxillofacial Surgery, St Richard's Trust, Chichester, West Sussex PO 19 4SE, UK; 3) Human and Clinical Genetics Unit, Molecular Medicine Centre, Western General Hospitals Trust, Edinburgh EH4 2XU, Scotland, UK; 4) Department of Histopathology, Imperial College School of Medicine at St Mary's. Norfolk Place, London W2 1PG, UK.

Cherubism is a rare autosomal dominant disease of childhood characterised by proliferative lesions within the mandible and maxilla leading to prominence of the lower face and appearances reminiscent of Renaissance cherubs. Resolution of these bony abnormalities is often observed after puberty. Using two families with clinically, radiologically and/or histologically proven Cherubism, we have performed a genome wide linkage search and localised the gene to chromosome 4p16.3 with a maximum multipoint LOD score of 5.64. Both families showed evidence of linkage to this locus. Critical meiotic recombinants place the gene in a 3cM interval between D4S127 and 4p-telomere. Within this region a strong candidate is the gene for fibroblast growth factor receptor 3 (*FGFR3*), mutations in which have been implicated in a diverse set of disorders of bone development. Having screened a total of 7 families of cherubism by conformation-sensitive gel electerophoresis (CSGE), we have found no evidence of mutations within *FGFR3*. We also found no evidence for loss of heterozygosity (LOH) within this region when we analysed 13 mandibular biopsies with the characteristic features of cherubism from 8 affected individuals, indicating that cherubism is unlikely to be due to the inactivation of a tumor supressor gene. We are currently in the process of collecting further families, so that we can perform allelic association studies on approximately 20 families.

Program Nr: 2461 from the 1999 ASHG Annual Meeting

Faster multipoint linkage analysis through state space reduction. *K.G. Markianos, L. Kruglyak, M.A. Eberle.*
Molecular Medicine, Fred Hunchinson Cancer Research Center, Seattle, WA.

Computational constraints currently limit exact multipoint linkage analysis to pedigrees of moderate size. We are developing new algorithms that allow analysis of larger pedigrees by reducing CPU and memory requirements of the computation. The algorithms are being implemented in a new version of the software package GENEHUNTER. One algorithm uses the observed pedigree genotypes to reduce the inheritance vector space. Performing the calculation in the reduced vector space increases the efficiency of multipoint analysis several times both in terms of computational speed and memory requirements. Specifically, we introduce the following improvements:

1) Identification of the COMPLETE set of illegal inheritance vectors.
2) Computation of prior probabilities only for legal inheritance patterns.
3) Efficient computation of the Whittemore & Halpern statistic used for nonparametric (NPL) linkage analysis.
4) Computation and storage of cumulative transition probabilities only for states compatible with the observed genotypes.

We will present performance data for the new version of GENEHUNTER.

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Program Nr: 2462 from the 1999 ASHG Annual Meeting

A weighted sibship disequilibrium test for linkage and association in discordant sibships. *E.R. Martin[1], S.A. Monks[2], N.L. Kaplan[3].* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Department of Biostatistics, University of Washington, Seattle, WA; 3) Biostatistics Branch, NIEHS, Research Triangle Park, NC.

For a data set consisting of discordant sib pairs, with one affected and one unaffected child, Horvath & Laird (1998) presented two statistics that can be used to test the null hypothesis of no linkage or no association between a biallelic marker and a disease locus. For a discordant sib pair, let d denote the difference between the number of copies of marker allele 1 in the affected sib and unaffected sib. T_1 is based on sgn(d), where sgn(d) is +1 or -1 depending on whether d is positive or negative, while T_2 is simply based on d. In a sample of discordant sib pairs the test with T_2 is the same as both Curtis's test and the S-TDT (Curtis 1997, Spielman & Ewens 1998). Under the null hypothesis, both statistics are asymptotically normal with mean 0 and variance 1. Horvath & Laird (1998) showed that, when testing for association in the presence of linkage, the two tests have similar power in most situations, with neither test uniformly most powerful. These statistics can also be used to construct tests for association and linkage in larger sibships. For a larger sibship, Horvath & Laird (1998) defined $d = m_A - m_U$, where m_A and m_U are the mean numbers of copies of marker allele 1 in the affected and unaffected sibs, respectively. With this more general definition of d, their sibship disequilibrium test (SDT) uses T_1. Just as for sib pairs, a test could also be based on T_2, and we refer to the test as the weighted sibship disequilibrium test (WSDT), since the sign of the difference is weighted by the magnitude of this difference in the calculation of T_2. We have conducted computer simulations to examine the power of the WSDT. Our simulation results suggest that, unlike for sib pairs, for larger sibships the test with T_2 is often more powerful than the test with T_1 when testing for association in the presence of linkage, particularly when sibships contain multiple affected siblings. We also discuss extensions of the WSDT to multiallelic markers and multiple marker loci.

Program Nr: 2463 from the 1999 ASHG Annual Meeting

Likelihood methods for model-free linkage analysis of multiple-affected sibships. *M.M. Martinez, Z. Uhry, F. Demenais.* Genetique des Maladies Humaines, Hopital St Louis, INSERM U358, Paris, France.

Several likelihood methods have been proposed to test for linkage using Affected Sib-Pairs. The ASP-MLS (Risch 1990, Holmans 1993) method maximizes the likelihood as a function of two zj's parameters (probability for a sib-pair to share j marker alleles Identical By Descent). The distribution of the statistic, 2ln(10)MLS, is a mixture of chi-squares with 1 and 2 df. For sibships with multiple affected sibs, type I and type II errors of the ASP-MLS statistic are not well defined. An other approach, recently proposed, can be applied to the whole sibship of affecteds (Terwilliger 1994; Abel 1998). The statistic (MLB) is assumed to be a linear function of y (IBD sharing rate). The MLB approach is equivalent to the lod score maximized as a function of r (recombination fraction between the disease and the marker locus), assuming a recessive mode of transmission with complete penetrance and no phenocopies (f2=1, f1=f0=0) and setting parental phenotypes to unaffected (Knapp 1994). We propose the Z-mod likelihood method to test for linkage without linear constraint (as with the general MLS model) while analyzing sibships of affected sibs. The Z-mod is the lod score function maximized as a function of the genetic parameters (q, f2, f1 and f0) with r=0 and with parental phenotypes being set to unknown. We investigated the statistical distribution of the Z-mod when families have s=2, 3 or 4 affected sibs. We computed the expected maximum of Z-mod and MLB statistics and their exact sample distributions for different sample sizes (n) of families, under different hypotheses on linkage (r=0.5 vs. 0) and on the effects of the disease locus. We considered a fully informative marker. For families with two affected sibs only, Z-mod is equivalent to the MLS method. For the genetic effects considered, the linkage test has greater power using Z-mod than using MLB methods, particularly when s increases. The Z-mod approach can be easily generalized to non-parametric linkage analysis incorporating covariate effects. These situations will be further explored by Monte-Carlo simulations.

Program Nr: 2464 from the 1999 ASHG Annual Meeting

MAP-O-MAT: marker-based linkage mapping on the World Wide Web. *T.C. Matise, J.A. Gitlin.* Lab of Statistical Genetics, Rockefeller University, New York, NY.

Genetic linkage maps are commonly used in many types of genetic analyses, including parametric and non-parametric linkage analyses searching for genes for both simple Mendelian as well as complex, oligogenic traits. Although 2-5 cM linkage maps have been available for over 3 years, no single map contains all available polymorphic markers. In addition, on any map only a portion of the markers are placed in single map positions with high statistical support while most can only be localized to larger bins. Therefore, it can still be a difficult and tedious exercise to determine the order and map distances for any given set of polymorphic markers.

We have developed a Web-based server (MAP-O-MAT) for automated linkage mapping of polymorphic DNA markers. MAP-O-MAT facilitates verification of order and map distances for custom mapping sets, eliminating the need to download genotype data and compute likelihoods locally. MAP-O-MAT uses the CEPH genotype database version 8.2 comprising nearly 12,000 markers scored in the CEPH reference pedigrees. The CRI-MAP program (Lander and Green, 1987) is used for likelihood calculations and some mapping algorithms. For any given set of ordered markers, MAP-O-MAT can compute map distances using either the Haldane or Kosambi map functions, run CRI-MAP's FLIPS analysis to evaluate local support for order, and can determine the location and corresponding statistical support of additional markers on the given map. MAP-O-MAT also allows evaluation and comparison of statistical support for multiple user-specified marker orders. MAP-O-MAT has a special feature which localizes marker(s) of interest on the Genemap '99 framework map, thus facilitating identification of regions of interest on the Genemap. In addition, MAP-O-MAT provides marker-specific information not easily found elsewhere, including observed heterozygosity, the number of families genotyped, the number of meioses, the number of informative meioses, and the number of phase-known meioses available for each marker. MAP-O-MAT is a Java servlet and is located at http://linkage.rockefeller.edu/tara/mapomat. Development of MAP-O-MAT is funded by NIH grants R29HG01691 and HG00008.

Program Nr: 2465 from the 1999 ASHG Annual Meeting

Consistency of nominal and empirical p-values in QTL linkage analysis. *R.C. McEachin, W.L. Duren, R.M. Watanabe, M. Boehnke, The FUSION study group.* Dept Biostatistics, Univ Michigan, Ann Arbor, MI.

Quantitative trait locus (QTL) linkage analysis is a tool for localizing genes responsible for quantitative traits. Variance components methods provide a powerful approach to QTL analysis, using intact pedigrees. Given independent pedigrees of identical structure, normally-distributed data, and observed identity-by-descent states for all pairs of family members, QTL LOD scores are asymptotically distributed as a 1/2:1/2 mixture of chi-square on one degree of freedom and point mass at zero, under the null hypothesis of no linkage. Given finite samples and the likely violation of most of these assumptions, we tested how well this distribution fits actual data. We examined results from the FUSION study, in which we are trying to map and identify genes for type 2 diabetes and diabetes-related quantitative traits. For each of 39 analyses in which we obtained a QTL LOD score ³ 2.5, we used computer simulation to assess empirical p-values (Pe). In these simulations, we used the same family structures, genetic maps, and markers as in FUSION. For each of 10,000 or 100,000 replicate samples, we simulated marker genotypes for all family members, assuming that no trait-affecting gene was present. We then estimated Pe as the proportion of replicates yielding LOD scores at least as large as we observed in the real data. We compared the resulting Pe values to the nominal p-values (Pn) obtained under the assumed mixture distribution. In 36 of the 39 analyses, Pn was within the 95% exact binomial confidence interval for Pe. Of the three outside the interval, two were on the boundary. On average, the Pe values were slightly larger than Pn, suggesting that the nominal p-value tends to overstate slightly the true linkage evidence. We note that for all 39 analyses, we transformed the data to approximate univariate normality prior to QTL linkage analysis. Without this step, the nominal p-values likely would have been far less accurate. These results suggest that using the nominal p-value in an initial screen is appropriate but, for results of interest, computer simulation to assess the actual significance level should be used.

Program Nr: 2466 from the 1999 ASHG Annual Meeting

Refinement of the genetic mapping of the hemochromatosis gene by use of haplotype sharing analysis, and application of this method to Multiple Sclerosis. *G.J.te Meerman[1], I.M. Nolte[1], G.T. Spijker[1], M. Boon[1,2]*. 1) Medical Genetics, University of Groningen, the Netherlands; 2) Department of Neurology, University Hospital Groningen, the Netherlands.

Thomas et al. (1998) and Ajioka et al. (1997) have analyzed the single and multilocus marker association between repeat markers and the hemochromatosis gene. It appeared that the marker that was most closely linked was less associated with the disease than markers at greater distance from the gene. One reason that there may be a discrepancy between the strength of association and linkage is that a mutation occurs on a chromosome with a unique marker allele that is further away than a nearby marker allele that occurs without the mutant allele. Especially with SNPs such a situation is likely. If marker alleles are used that are in linkage disequilibrium haplotype agreement will be an indication for identity by descent. Analyzing the overlap between all pairs of haplotypes will then give an indication for the identity by descent status of intervals between markers. The interval where the probability of identity by descent has the strongest contrast between cases and controls will then most likely contain the mutant allele(s). We applied a method of haplotype by haplotype comparison to the data sets of Thomas and Ajioka and found that the most linked marker allele was indicated. We also applied this method to our own data on Multiple Sclerosis and found that while the association analysis was inconclusive with respect to the gene position only one marker interval was indicated to harbour mutant alleles. Ajioka, R.S. et al. Haplotype analysis of hemochromatosis: evaluation of different linkage-disequilibrium approaches and evolution of disease chromosomes. Am. J. Hum. Genet. 60, 139-1447 (1997) Thomas, W. et al. A haplotype and linkage disequilibrium analysis of the hereditary hemochromatosis gene region. Hum. Genet. 102, 517-525 (1998).

Program Nr: 2467 from the 1999 ASHG Annual Meeting

Genetic studies on generalized Osteoarthritis. *I. Meulenbelt[1, 2], C. Bijkerk[3, 1], J.J. Houwing-Duistermaat[3], F.C. Breedveld[2], C.M. van Duijn[3], P.E. Slagboom[1].* 1) Vascular/Connective Tissue Res, TNO-Prevention & Health, Leiden, Z-H, Netherlands; 2) Dept. of Rheumatology, Leiden University Medical Center, Leiden; 3) Dept. of Epidemiology & Biostatistics Erasmus University Medical School, Rotterdam.

Osteoarthritis (OA) is characterized by degradation of articular cartilage and formation of new bone. Previously, genetic linkage analysis was performed a Dutch family in which primary generalized OA (GOA) without dysplasia occurred at an early age of onset (20-35 years). Most candidate genes that were tested in this family were excluded to cause the development of GOA (1). Among these loci, were genes encoding matrix components (COL2A1, COL9A1, COL9A2, COL11A1, COL11A2, COMP, and CRTL-1), and factors involved in cartilage remodeling (MMP3). To identify new OA genes a genome wide search was performed using this and 3 additional Dutch families. In all families OA occurred at early ages of onset in multiple joint sites simultaneously and was inherited in a dominant Mendelian fashion. Together these families consisted of 75 individuals of which 35 were affected. A genome wide search was performed using 300 Weber markers from set 8/8a with an 18-cM spacing. Linkage was observed only for a single chromosomal region with a maximum LOD-score of 2.2. Our genome wide search indicates a chromosomal region that may contain a gene involved in early onset generalized OA. 1) Meulenbelt I. et al. (1997) J Med Genet 34:1024-1027.

Program Nr: 2468 from the 1999 ASHG Annual Meeting

Non-hyperandrogenic polycystic ovarian syndrome is associated with short CAG repeats in the androgen receptor. *A. Mifsud[1], B. Rodrigez[2], E.L. Yong[1]*. 1) Obstetrics and Gynaecology, National University of Singapore, Singapore; 2) Pedriatics, National University of Singapore, Singapore.

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine disorder, which is considered to be the commonest cause of anovulatory infertility. The mode of inheritance of PCOS is uncertain but the disease is clustered in families. Although PCOS is responsible for at least 85% of women with hirsutism the percentage of hirsute women with elevated levels of testosterone seems to be lower. The mode of action of androgens is in conjunction with the AR. The exon 1 of the AR contains a polymorphic region with a variable number of CAG repeats. We looked at the role that the number of CAG repeats in the AR plays in the etiology of PCOS. We have examined the CAG repeat segment from 91 patients with ultrasound diagnosis of PCOS associated with irregular infrequent menstrual cycles and compared them to 112 controls of proven fertility and who have normal regular cycles. Patients were 83% Chinese and 17% Indians while that of the controls were 92% Chinese and 8% Indians. We found no differences between the mean CAG repeats length in patients compared to controls being the mean 22.97 and 23.09 respectively. Because each female has two X-chromosomes we then performed the analysis on the short and long alleles separately. Since there is a subset of PCOS whose androgens are normal we wondered whether there were any differences in CAG length between those with normal or low T compared to those whose T was high. The mean T in the normal population was 0.5 ng/ml. We divided the patients into two groups, those with T less than 0.5 and those with T more than 0.5. There was difference in the short alleles in between patients with low T compared to those with high T ($p=0.004$). There was no difference in the long alleles of patients with low or high T. There were still differences in the short CAG allele when Chinese and Indians patients were analyzed separately. There were also ethnic differences in our data. The mean of average CAG length in combined patients and controls were significantly different between for Chinese and Indians, being 23.16 and 22.08 respectively ($p=0.035$).

Program Nr: 2469 from the 1999 ASHG Annual Meeting

The gene for X-linked Vacuolated Myopathy (XVM), is located in a 14 cM span of Xq28. *BA. Minassian[1], MP. Auranen[2], SW. Scherer[1], M. Villanova[3], F. Muntoni[4], M. Fardeau[5], H. Kalimo[2].* 1) Departments of Pediatrics and Genetics, Hospital for Sick Children, Toronto, M5G 1X8, Canada; 2) Department of Human Molecular Genetics, National Public Health Institute, Helsinki, and Department of Pathology, Turku University, Finland; 3) Laboratorio di Patologia Neuromuscolare Istituto Ortopedico "Rizzoli",Bologna, Italy; 4) Department of Paediatrics & Neonatal Medicine, Hammersmith Hospital, London, UK; 5) Inserm U.153, Paris, France.

X-linked vacuolated myopathy (XVM), also known as X-linked myopathy with excessive autophagy (XMEA), was first described by Kalimo et al. in 1988. XMEA/XVM is characterized clinically by slowly progressive muscle weakness, mainly in lower limbs, and histopathologically by vacuolation of myofibers. The pathogenesis of this myopathy was suggested to be a sublethal intrinsic defect of myofibers which led to abundant autophagy as well as exocytosis of the phagocytosed debris, deposited between multiplied basement membranes around the injured myofibers (the intrinsic hypothesis). Since then, Muntoni et al. (1994) and Villanova et al. (1995) have described several other families and the latter showed that the vacuoles in this disease are unique among vacuolar myopathies in that they are lined by plasma and basement membrane and have strong deposition of the complement C5b-9 membrane attack complex (MAC). An alternate hypothesis was thus proposed whereby MAC attack causes invaginations of the plasma membrane leading to the formation of the vacuoles (the extrinsic hypothesis).

We have embarked on a project aimed at identifying the gene responsible for XMEA/XVM. We have so far recruited seven families three of which independantly prove linkage to chromosome Xq28. Based on a number of recombinations, we have been able to narrow the gene locus to 14 cM. At present, we are collecting new families and analyzing candidate genes for mutations. Identifying the gene responsible for XMEA/XVM and characterizing its protein product should provide important insights into the pathogenesis of this unique genetic form of vacuolar myopathies and into the physiology of muscle.

Program Nr: 2470 from the 1999 ASHG Annual Meeting

The -308 promoter polymorphism in the tumor necrosis factor-a **gene is associated with higher body mass index in white girls during adolescence.** *S.P. Moffett[1], S.Y.S Kimm[2], N.W. Glynn[2], C.E. Aston[1], R.E. Ferrell[1].* 1) Dept. of Human Genetics, Univ of Pittsburgh, Pittsburgh, PA; 2) School of Medicine, Univ of Pittsburgh, Pittsburgh, PA.

Tumor necrosis factor-a (TNF-a) is a modulator of gene expression in adipose and muscle tissues. Elevated levels of TNF-a are implicated in the development of both cachexia (loss of muscle and adipose tissue seen in inflammatory states and cancer) and obesity. This report examines the effect of variation in the TNF-a gene on measures of body composition in a biracial cohort of girls followed from ages 9-10 (Yr 1) to 18-19 (Yr 10). Body composition measures included body mass index (BMI, kg/m^2), sum of skinfolds at the triceps, subscapular and suprailiac sites (SSF) and lean body mass (LBM, assessed by bioelectrical impedance). The -308 TNF-a polymorphism, a G (TNF*1) to A (TNF*2) substitution in the promoter region reported to alter TNF-a expression, was typed in 642 black girls and 641 white girls participating in the NHLBI Growth and Health Study, a longitudinal study of obesity development during adolescence. Allele frequencies did not differ between the two races (q=0.181 in black girls; q=0.176 in white girls) and were in Hardy-Weinberg equilibrium. Cross-sectional analysis by age showed a significant association between the TNF 2-2 genotype and higher BMI in white girls (TNF 1-1=23.1 vs. TNF 2-2=25.4, p=0.0085 at Yr 10). Also, those with the TNF 2-2 genotype had a 4 kg greater LBM compared to TNF 1-1 or 1-2 (Yr10, p=0.0004). No such relationship was seen in black girls. No significant association was found between TNF-a genotype and SSF in either group. In summary, these results show a significant association between the -308 TNF-a polymorphism and BMI in white girls. Since no associations were found between TNF-a and body fat, the association with BMI is most likely a reflection of the effect of TNF-a on LBM.

Program Nr: 2471 from the 1999 ASHG Annual Meeting

Hepatocyte Nuclear Factor-6: Studies of Associations between Genetic Variability and Type 2 Diabetes or Estimates of Insulin Secretion. *A.M. Moller[1], J. Ek[1], S. Durviaux[2], S.A. Urhammer[1], J.O. Clausen[1], H. Eiberg[3], T. Hansen[1], G.G. Rousseau[2], F.P. Lemaigre[2], O. Pedersen[1].* 1) Steno Diabetes Ctr, Copenhagen, Denmark; 2) Hormone and Metabolic Research Unit, Université Catholique de Louvain and Christian de Duve Institute of Cellular Pathology, B-1200 Brussels, Belgium; 3) University Institute of Medical Biochemistry and Genetics, University of Copenhagen, Copenhagen, Denmark.

Since the transcription factor hepatocyte nuclear factor (HNF)-6 is an upstream regulator of several genes involved in the pathogenesis of maturity-onset diabetes of the young (MODY), we have tested the hypothesis that variability in the HNF-6 gene is associated with subsets of type 2 diabetes or estimates of insulin secretion. We cloned the coding region as well as the intron-exon boundaries of the two exons of the human HNF-6 gene. We then examined them on genomic DNA in six MODY probands without mutations in the MODY1, MODY3 and MODY4 genes and 54 patients with late-onset type 2 diabetes by combined SSCP-heteroduplex analysis followed by direct sequencing of identified variants. We found two silent variants (Pro94Pro, Gly287Gly) and one missense variant (Pro75Ala). In an association study the allelic frequency of the Pro75Ala polymorphism was 3.2% (95% confidence interval, 1.9-4.5) in 330 type 2 diabetic patients and 4.2% (2.4-6.0) in 238 age-matched glucose tolerant control subjects. There were no differences between carriers (N=19) and noncarriers (N=219) of this polymorphism in glucose-induced serum insulin and C-peptide release during an oral glucose tolerance test in 238 middle-aged, glucose tolerant subjects. Moreover, in genotype-phenotype interaction studies of 226 glucose tolerant off-spring of type 2 diabetic patients and of 367 young healthy subjects, the carriers (N=21 and N=15, respectively) of the polymorphism did not differ from non-carriers (N=205 and N=352, respectively) in acute (0-8 min) serum insulin or C-peptide responses during an intravenous glucose tolerance test. In conclusion, mutations in the coding region of the HNF-6 gene are not associated with type 2 diabetes or with changes in insulin secretion among the Caucasians examined.

Program Nr: 2472 from the 1999 ASHG Annual Meeting

Dizygotic twinning is not linked to variation at the a**-inhibin locus.** *G.W. Montgomery[1], D.L. Duffy[1], B.R. Haddon[1], M. Kudo[2], E.A. McGee[2], J.S. Palmer[1], A.J. Hsueh[2], D.I. Boomsma[3], N.G. Martin[1].* 1) Genetic Epidemiology Unit, Queensland Institute of Medical Research and Joint Genetics Program, The University of Queensland, Brisbane, Queensland, Australia; 2) Division of Reproductive Biology, Department of Gynecology and Obstetrics, Stanford University Medical Center, USA; 3) Psychology Department, Free University, Amsterdam, Netherlands.

Natural multiple pregnancy in women leading to dizygotic (DZ) twins is familial and varies across racial groups suggesting a genetic predisposition. Mothers of DZ twins have a higher incidence of spontaneous multiple ovulation and several studies report elevated follicle-stimulating hormone (FSH) concentrations in mothers of twins. Release of FSH is controlled by feedback of inhibin peptides from the ovary. Inhibin peptides are heterodimers of a common a-subunit and either bA- or bB-inhibin subunits. Immunization against inhibin a-subunit results in increased ovulation rate in animals and the inhibin a-subunit is therefore a candidate gene for mutations that may increase the frequency of DZ twinning.

A C/T polymorphism at base pair 128 was identified in exon 1 of the human a- inhibin gene. Restriction digests of a PCR product from exon 1 with the enzyme *Spe* I detects two alleles of 447 bp and 124 bp. The polymorphism was typed in 1328 individuals from 325 pedigrees of sister pairs that all had at least one set of DZ twins. The frequencies for the 447 bp and 124 bp alleles were 0.802 and 0.198 respectively. Analysis using a dominant model excluded linkage between the a- inhibin locus and DZ twinning in this population (LOD score -2.81 at theta equals zero). TDT and case control based association tests were also negative. We conclude that dizygotic twinning is not linked to variation in the a-inhibin locus.

Program Nr: 2473 from the 1999 ASHG Annual Meeting

Childhood absence epilepsy in 8q24: haplotypes, recombinations, and construction of physical map. *R. Morita[1,2], Y. Sugimoto[2], G.C.Y. Fong[1], P.U. Shah[3], I.P. Castroviejo[4], S. Khan[1,5], K. Yamakawa[2], A.V. Delgado-Escueta[1]*. 1) Epilepsy Genomics laboratories, Comprehensive Epilepsy Program, UCLA School of Med and West L.A. DVA Medical Center, Los Angeles, CA; 2) Lab for Neurogenet, Brain Sci Inst, The Inst of Physical & Chemical Res, Saitama, Japan; 3) K.E.M. Hospital & Seth G.S. Medical College, Bombay, India; 4) Pediatric Neurology University Hospital La Paz, Madrid, Spain; 5) Neurosciences Dept, Riyadh Armed Forces Hospital, Riyadh, Saudi Arabia.

Childhood Absence Epilepsy (CAE) is a common form of idiopathic generalized epilepsy accounting for 5 to 15% of all epilepsies. In 1998, we mapped the locus responsible for CAE and tonic-clonic seizures to chromosome 8q24 (*ECA1*; MIM No. 600131) by linkage analysis using a five-generation family from India and five medium sized multiplex families from USA, Argentina, Spain, and Saudi Arabia (Fong et al., 1998). Haplotype analyses suggested a 3.2 cM interval flanked by D8S1710 and D8S502. We excluded two candidate genes, *KCNQ3*(voltage gated potassium channel) and *JRK* (human homologue of mouse jerky) located on 8q24 by their location and mutation analyses (Morita et al., in press). As a further step to isolate the *ECA1* gene, we screened a BAC library with 16 STS markers on 8q24 and expanded the contigs by BAC end walking to create a physical map, composed by 17 BACs and two YACs. These clones spanned about 3Mb from D8S1710 to D8S523, and provided the correct order of markers around the *ECA1* gene. We used the physical map to reorder marker positions, and further studied haplotypes and recombinations in CAE families with additional markers. We identified consanguinity in the first and second generations of the Indian family. Both individuals of the first generation were first cousins and one of their sons married his aunt. Consanguinity and identical haplotypes suggested all relatives in the first and the second generations had the same *ECA1* mutation that segregated with affected members through five generations. Our present results reduced the size of *ECA1* region to 700kb bounded by D8S1753 and D8S502. Large scale sequencing of BACs is on going looking for candidate genes for *ECA1*.

Program Nr: 2474 from the 1999 ASHG Annual Meeting

Mega2, a data-handling program for facilitating genetic linkage and association analyses. *N. Mukhopadhyay[1], L. Almasy[1], M. Schroeder[2], W.P. Mulvihill[3], D.E. Weeks[1]*. 1) Human Genetics, University of Pittsburgh, Pittsburgh, PA; 2) Stanford University, Stanford, CA; 3) Carleton College, Northfield, MN.

During a linkage analysis project, it can be quite difficult to get one's data in the proper format required by each different computer program. Not only must the data be converted to the proper format, but also the loci must be reordered into their proper order. To address this problem, we created Mega2.

A typical linkage-analysis study requires information regarding pedigree structure, trait phenotypes, and genetic marker data. One commonly-used data format is the one used by the LINKAGE programs. LINKAGE-format files come in pairs: the "datain.dat" contains the locus information (disease model, allele frequencies, numbers of alleles, etc.), while the "pedin.dat" contains the pedigree structure information and phenotypes. However, the original LINKAGE format made no provisions for locus names nor for marker map information. So Mega2 uses as input a trio of files which remedy these omissions: 1) the locus file modified to contain locus name information; 2) the pedigree file; and 3) the map file. Mega2 then takes this trio of input files and, via a menu-driven interface, transforms them into various other file formats, thus greatly facilitating a variety of different analyses. In addition, for many of these options, it also sets up a C-shell script that can then automatically run these analyses. Programs that are currently supported include SIMWALK2, MENDEL, ASPEX, APM, SLINK, SPLINK, SIMULATE, SAGE, GeneHunter, GeneHunter-Plus, TDTMax, SOLAR, and Guo and Thompson's program for testing for Hardy-Weinberg equilibrium. In addition, Mega2 can generate summaries about pedigree structures and allele frequencies, as well as can convert larger pedigrees to their component nuclear families.

Mega2 currently uses a textual interface. We plan to use it as the engine behind some more graphical interfaces under development. Mega2 is available via our web site http://watson.hgen.pitt.edu/.

This work was supported in part by the following NIH grants: HG00932, AG16989, AG16992, EY09859, and DK55406.

Program Nr: 2475 from the 1999 ASHG Annual Meeting

The TDT/sib-TDT procedure applied to the study of the 5HT1Dbeta receptor gene and Obsessive-Compulsive Disorder. *E. Mundo[1], M.A. Richter[2], K. Hood[2], F. Sam[1], F. Macciardi[1], J.L. Kennedy[1].* 1) Neurogenetics Section, CAMH,University of Toronto, Toronto, Ontario, Canada; 2) Anxiety Disorders Clinic, CAMH,University of Toronto, Toronto, Ontario, Canada.

Obsessive-Compulsive Disorder (OCD) is a complex psychiatric disease with a strong genetic component (Pauls et al, 1995; Billet et al, 1998). Data from challenge studies and from pharmacological trials have pointed to the involvement of serotonin (5HT) in the pathogenesis of OCD (Barr et al, 1992; Mundo et al, 1995, 1999). As for other complex diseases, investigating for the presence of linkage disequilibrium is a particularly useful strategy in identifying genes that may contribute to disease susceptibility, particularly when genes are of small effect. Recently an extension of the Transmission Disequilibrium Test (TDT) (Spielman et al, 1993) has been developed, the sib-TDT (Spielman and Ewens,1998), which compares marker genotypes in affected and unaffected offspring. The combination of the two procedures in one test, named TDT/sib-TDT (Spielman and Ewens, 1998) allows the incorporation of families with known parental genotypes together with families having known unaffected sibs' genotypes and unknown parental genotypes. We applied this procedure to a set of data from 67 probands diagnosed as affected by OCD according to DSM-IV criteria (APA, 1994) and their living parents or siblings, genotyped for the G861C polymorphism of the 5HT1Dbeta autoreceptor gene (Lappalainen et al, 1995). This receptor is particularly interesting in OCD as data from recent studies have shown that the 5HT1Dbeta selective ligand sumatriptan may improve symptoms in OCD patients resistant to conventional pharmacotherapy (Stern et al, 1998). Thirty-two families were informative for the analysis. Results showed a preferential transmission of the G allele to the affected subjects ($z=2.524$, $p<.01$). Our findings need to be replicated on larger samples. If confirmed, the 5HT1Dbeta receptor gene will be implicated in either the pathogenesis of OCD, or in the mechanism of the pharmacological response.

Program Nr: 2476 from the 1999 ASHG Annual Meeting

Silver syndrome, a distinct form of hereditary spastic paraplegia (HSP), is not linked to any of the known HSP loci. *V.A. Murday[1], H. Patel[1], P. Hart[2], T. Warner[2], I. Allen[1], H. Phillimore[1], M. Patton[1], A.H. Crosby[1]*. 1) Dept Medical Genetics, St Georges Hosp Medical Sch, London, England; 2) Dept Neurology, Royal Free Hospital, London, England.

The hereditary spastic paraplegias are a clinically and genetically diverse group of disorders which share the primary feature of a progressive and severe lower extremity spasticity. The HSPs have been divided into 'pure' and 'complicated' forms depending upon the absence or presence of secondary neurological abnormalities. Silver syndrome is a particularly disabling autosomal dominant form of complicated HSP in which the leg spasticity is associated with wasting of the hand muscles. In view of the fact that genes for hereditary spastic paraplegia can produce highly variable phenotypes, the five known autosomal dominant loci were investigated for linkage to Silver syndrome. However, genotyping of these loci in 2 large multigeneration families was incompatible with linkage to any of these regions, suggesting that an additional locus is responsible for this syndrome.

Program Nr: 2477 from the 1999 ASHG Annual Meeting

Fine-scale mapping of the Congenital Fibrosis of the Extraocular Muscles type 2 (CFEOM2) gene region. *M. Nakano, E.C. Engle.* Dept Genetics, Children's Hospital, Boston, MA.

The syndromes known as congenital fibroses of the extraocular muscles (CFEOM) are congenital ocular-motility disorders manifested as restrictive ophthalmoplegias with ptosis. CFEOM2 (MIM 602078) is an autosomal recessive form of this disorder characterized by bilateral ptosis, with the eyes frozen in an exotropic (outward) position. Three consanguineous Saudi Arabian families have been identified with CFEOM2, and we have previously mapped their disease gene to a 2.5 cM region of distal 11q13 between D11S4162 and D11S4184. Two of the three families share a common disease-associated haplotype, suggesting a founder effect for CFEOM2.

Toward our goal of narrowing the critical region and cloning the CFEOM2 gene, we have constructed a BAC contig across the ~1300kb CFEOM2 critical region. This map contains 40 BAC clones with a minimal tiling path of 6 BACs. Through di- and tetra-nucleotide repeat oligonucleotide hybridization we have, to date, identified 25 new short tandem repeat polymorphisms in the region and have mapped them onto the BAC contig. We are currently testing these markers for recombination events in family members and for evidence of a common haplotype shared by all three CFEOM2 families. In addition, we have mapped 15 new EST/genes to the region, in addition to the 5 genes already present on the existing YAC map. These ESTs/genes, as well as any others identified by exon trapping, will be analyzed as candidate CFEOM2 disease genes.

Although the CFEOM syndromes are thought to arise from dysfunction of the oculomotor nerve, their molecular etiology remains unknown. Identifying the CFEOM2 gene will reveal the genetic mechanism underlying this recessive form of CFEOM, and should be valuable to our understanding of the CFEOM disease process and the role these genes play in the normal development of the oculomotor system. In addition, this relatively gene rich region of distal 11q13 is not currently covered by the Human Genome Project, and thus our positional cloning project will contribute to this larger effort.

Program Nr: 2478 from the 1999 ASHG Annual Meeting

Genome Coverage and Fidelity of Whole Genome Amplification. *K.C. Neal, A.C. Lidral.* Orthodontics, Ohio State University, Columbus, OH.

For most studies of human genetics, sample DNA is a limiting resource, particularly when tissue or blood samples are minimal. In these cases, the amount of DNA restricts analysis to a small number of experiments, thus preventing genome wide linkage or linkage disequilibrium mapping. In addition, with the advent of SNP's a larger amount of initial template will be necessary to permit genome wide coverage. A variety of whole genome amplification (WGA) techniques have been developed to circumvent this problem. These include inter-Alu PCR and a variety of degenerate oligonucleotide PCR's. Previous studies using WGA have shown complete genome coverage at cytogenetic resolution. However, to date, there are no comprehensive studies to address the issues of genome coverage or amplification fidelity to validate the use of WGA for mapping and sequencing purposes. The purpose of this study was to evaluate the genome coverage and the loss of heterozygosity (LOH) following WGA. DNA from 90 CEPH individuals was subjected to WGA performed by a modified degenerate oligonucleotide primed PCR protocol of Zhang et al. (PNAS 89:5847, 1992). The amplified DNA was subsequently genotyped using a low density set of microsatellite markers from the Weber 8 Mapping Panel. Genotyping results were compared to published genotypes for the CEPH individuals (www.cephb.fr). Preliminary analysis of 8 markers from 5 chromosomes revealed a loss of heterozygousity (LOH) rate of 5.3% with the loss of larger alleles being more frequent. The LOH rate was inconsistent with several markers giving higher rates while others were reasonably low. The LOH rates were not correlated with the size or sequence of the repeat marker. The percentage of samples with incorrect genotypes compared to published results for CEPH individuals was 0.8%. The percentage of sample amplification was 97.1% and 1.3% of the amplified samples were too ambiguous to be scored which is comparable to published results. This preliminary data suggests that WGA exhibits a variable rate of fidelity that may prove to be acceptable for a majority of markers. Experiments are ongoing to determine if the coverage and fidelity of WGA would permit genotyping using a 20 cM mapping panel.

Program Nr: 2479 from the 1999 ASHG Annual Meeting

***NIDDM1* is associated with diabetes and body fat distribution in the Hutterites.** *D.L. Newman[1], A. Tsalenko[1], N.J. Cox[1,2], C. Ober[1]*. 1) Department of Human Genetics, University of Chicago, Chicago, IL; 2) Department of Medicine, University of Chicago, Chicago, IL.

Non-insulin-dependent diabetes (NIDDM) is a complex disease with widespread health implications. Hanis et al. (1996, Nat Genet 13:161-166) reported significant evidence for linkage between NIDDM in Mexican-Americans and a marker at 2qter (*NIDDM1*). A single variable site, SNP43, completely partitioned the evidence for linkage in those data. Here we report that the *NIDDM1* locus is also associated with NIDDM in the Hutterites of South Dakota, an inbred Caucasian population. We examined SNP43 as well as a variable site 11 bp away, SNP44, using the TDT. The common allele of SNP44 (T) was overtransmitted to affected individuals (15T:3U), while the common allele of SNP43 (G) did not show significant excess transmission (27T:22U). The common SNP44-SNP43 haplotype (T-G) was overtransmitted (42T:27U) while the two other observed haplotypes (T-A and C-G) were undertransmitted (24T:30U and 7T:16U, respectively) to affected individuals, suggesting that both SNPs contribute to NIDDM susceptibility. In addition, although the T-G haplotype was associated with lower BMI and percent body fat, females homozygous for the common variant of SNP44 (T/T) or carrying the common haplotype (T-G) had a higher mean waist-to-hip ratio ($P = 0.018$ and $P = 0.013$, respectively). A high waist-to-hip ratio is a significant risk factor for developing NIDDM. Overall, these data are consistent with the hypothesis that variation at *NIDDM1* influences both diabetes susceptibility and body fat distribution in the Hutterites. (Supported by NIH grants HL56399 and HL07237.).

Program Nr: 2480 from the 1999 ASHG Annual Meeting

Localization of FEVE (Familial Enteropathy with Villous Edema) to Human Chromosome 11 (11q23.1). *A.Y. Ng[1], M. Hicks[1], S.M. Haase[1], T. Johnson[1], J.S. Bamforth[1], H.F. Pabst[2], M.A. Walter[1], K.A. Sprysak[1], B.G. Elyas[1], L.M. Vicen-Wyhony[1], M.J. Somerville[1]*. 1) Medical Genetics, University of Alberta, Edmonton, Alberta, Canada; 2) Pediatrics, University of Alberta, Edmonton, Alberta, Canada.

Familial enteropathy with villous edema (FEVE [MIM 600351]) is an autosomal dominant disorder with variable penetrance (Smith et al., 1994). It is characterized by episodes of life-threathening secretory diarrhea with persistent jejunal histologic abnormalities associated with IgG2 deficiency. Patients present with a sudden onset of mild symptoms which include fever and occasional abdominal pain. These symptoms progress within 5 to 6 hours to intense nausea, persistent vomiting and very profuse watery diarrhea which leads to severe dehydration and hypovolemic shock. The age of onset of these symptoms varies from 6 months to 20 years and in all cases the episodes are less frequent in the teenage years with no family member having an episode after the age of 21 years. We have obtained blood samples from a large Mennonite kindred from Alberta, Canada which exhibits this syndrome. Two-point linkage analysis was conducted with a genome-wide screen for linked markers using the LINKAGE analysis program (Lathrop et al., 1985). DNA was genotyped on an Applied Biosytems model 377 (ABI PRISM Linkage Mapping Set Version 2; Perkin Elmer Applied Biosystems) using fluorescent-based microsatellite sets that included 400 markers defining a ~10 cM resolution human index map. 190 polymorphic markers from various panels were screened and a region on the long arm of chromosome 11 gave significant LOD scores. A maximum LOD score of 6.2 at theta = 0 was obtained with marker D11S908, and is localized to 11q23.1. This portion of chromosome 11 is currently under investigation with a higher density microsatellite array. Candidate genes in this region include: SCN2B, MMP12, CD3D, CD3E, CD3G, TAGLN, and IL10RA. Smith LJ, et al. (1994) J Pediatr 125: 541-548, Lathrop et al. (1985) Am. J. Hum Genet. 37: 82-498.

Program Nr: 2481 from the 1999 ASHG Annual Meeting

Association of neuropeptide Y receptor polymorphisms with obesity in a diabetic Ashkenaze population. *E.M. Nolin[1], T. Guillemette[1], J. Rennich[1], S. Lewitzky[1], X. Wang[1], J. Meyer[1], B. Glaser[2], M.A. Permut[3], A. Parker[1]*. 1) Human Genetics, Millennium Pharmaceuticals, Inc., Cambridge, MA; 2) Hadassah Medical Center, Jerusalem, Israel; 3) Washington University, St. Louis, MO.

The neuropeptide Y receptors (NPYR) located on chromosome 4q31-q32 are members of the G protein-coupled receptor superfamily and have been proposed as metabolic disease candidate genes owing to the role of NPY in appetite regulation. We investigated their association with non-insulin-dependent diabetes (NIDDM), an adult-onset polygenic disorder strongly associated with obesity. We screened NPY1R, NPY2R, and NPY5R for mutations using single-stranded conformation polymorphism (SSCP) and denaturing HPLC (DHPLC). Six polymorphisms were identified: in NPY1R, 2538 (A-G) in the 3' UTR and 640 (indel AAAT) 5' of the exon 1A promoter, in NPY2R, 2838 (A-G) in the 3'UTR and Ile195Ile (ATT-ATC) in exon 2, in NPY5R, Cys230Cys (TGT-TGC) and Gly426Gly (GGG-GGA) in exon 2. We typed four of these polymorphisms, 2538 (A-G) of NPY1R, 2838 (A-G) and Ile195Ile of NPY2R, and Gly426Gly of NPY5R, in a sample of Ashkenaze individuals collected in Israel, including 235 nuclear families characterized by one or more sibling pairs affected with NIDDM, 35 randomly ascertained trios, and 138 superunaffected controls (N=1074). We evaluated allelic and genotypic association of each marker with an obese diabetic phenotype. No significant association was observed, although genotypic associations with two markers, NPY1R 2538 (A-G) (p=0.102) and NPY5R Gly426Gly (p= 0.086), approached significance. There was no evidence of linkage disequilibrium between these two markers (p=0.49), nor association between two marker haplotypes and disease (p=0.24). We conclude that our sample shows no evidence of association between the neuropeptide Y receptors and obesity in the Ashkenaze diabetic population. This research was partially supported by Roche Pharmaceuticals, Inc.

Program Nr: 2482 from the 1999 ASHG Annual Meeting

Gilles de la Tourette syndrome: a replication study using family-based analyses of loci associated in an Afrikaner Population. *D.R. Nyholt[1], I. Simonic[2], G.S. Gericke[2], D. Gordon[1], J. Ott[1], J.L. Weber[3]*. 1) Laboratory of Statistical Genetics, Rockefeller University, New York, NY; 2) University of Stellenbosch, Matieland, South Africa; 3) Center for Medical Genetics, Marshfield Medical Research Foundation, Marshfield, WI.

Gilles de la Tourette syndrome (GTS) is a childhood-onset neurologic disorder characterized by motor and vocal tics and associated with behavioral abnormalities. Evidence supporting genetic determination of GTS comes from several lines of evidence including twin and family studies. However, gene-mapping efforts using large kindreds have generally proved unsuccessful. Subsequently, efforts are being redirected toward association studies in young, genetically isolated populations. Recently, we performed a genome scan using DNA samples from GTS patients and unaffected control subjects from the South Afrikaner population, and identified a number of regions significantly associated with GTS (Simonic et al. AJHG 63:839-846).

In the present study, five chromosomal regions previously showing evidence for association (p-values 10^{-1} to 10^{-6}), were examined in 91 unrelated nuclear families, using family-based tests for linkage and association. Single marker transmission disequilibrium (TDT) and haplotype relative risk (HRR) analysis (Terwilliger, AJHG 56:777-787) showed evidence for linkage or association with p-values of around 0.05. To further examine the presence of shared haplotypes among GTS individuals, two and three marker haplotypes were tested for excess transmission using an extended HRR contingency test, with significance determined by calculating exact p-values using Monte Carlo simulation. Extended haplotype analysis improved evidence for linkage disequilibrium at some regions ($P = 0.01$). As this study was designed to replicate previously significant results, our p-values of 0.05 or 0.01 represent significant replication and strong evidence for susceptibility loci in some genomic regions, although our sample size was relatively small.

Program Nr: 2483 from the 1999 ASHG Annual Meeting

Deterministic haplotyping algorithms for pedigree analysis and error checking. *J.R. O'Connell*[1,2]. 1) University of Pittsburgh, Pittsburgh, PA; 2) Wellcome Trust Centre for Human Genetics, University of Oxford, England.

As genetic maps using SNPs become more dense, researchers will incorporate more tightly spaced biallelic markers into their analyses, and thus increase the importance of haplotype information. Haplotypes allow geneticists to reconstruct gene flow within a pedigree, which can dramatically increasese the information content available for linkage analysis and positional cloning. Given a small region of the genome spanned by tightly linked markers of interest where no recombination is expected, we have developed a deterministic algorithm based on multilocus genotype elimination that can determine whether the pedigree has a haplotype configuration with zero recombinants. Moreover, if such a pedigree exists, then our algorithm can determine all such possible zero-recombinant haplotyped pedigrees. These configurations can then be used to estimate the haplotype frequencies for the pedigree data to test for linkage disequilibrium and also to perform a cladistic/measured haplotype analysis. The cladistic analysis constructs a haplotype tree to account for the most common haplotypes to try to find ancestral recombination events. Measured haplotype analysis then models the effect of a quantitative trait associated with particular haplotypes to estimate trait variability. Haplotype analysis can also be very useful for detecting the presence of genotyping errors in pedigrees that do not cause inconsistencies in Mendelian inheritance by detecting the presence of an excess of recombination events among closely spaced markers. We present a deterministic algorithm based on a technique called set-genotype elimimination that finds all consistent haplotype configurations for groups of two and three markers even in the presence of recombinants. In particular, if no zero-recombinant pedigree configuration exists, the algorithm can find pedigree configurations with the smallest number of recombinants and identify any haplotypes that contain obligate double recombinants. We present examples of our algorithms applied to real pedigree data. The work was supported in part by funds from NIH grant HG00932, NIA grant AG16992-01 and BIOMED EC grant PL 96 2532.

Program Nr: 2484 from the 1999 ASHG Annual Meeting

Analysis of Single Nucleotide Polymorphisms in Candidate Genes for Hypertension and Insulin Resistance. *M. Olivier[1,2], H.-H.W. Sheu[2], C.-Y. Jeng[2], C.-F. Hsiao[2], Y.-Z. Tseng[2], K. Ranade[1,2], Y.-D.I. Chen[2], R. Olshen[2], D. Curb[2], R. Pratt[2], N. Jarvis[3], M. De Arruda Indig[3], N. Risch[1,2], D.R. Cox[1,2]*. 1) Genetics, Stanford University, Stanford, CA; 2) SAPPHIRe Project; 3) Third Wave Technologies, Madison, WI.

The Stanford-Asian Pacific Program in Hypertension and Insulin Resistance (SAPPHIRe) is collecting Chinese and Japanese sib-pairs to map major genetic loci underlying hypertension. To date, 1,263 sib-pairs between 35 and 60 years have been collected. The cohort consists of 686 concordant hypertensive pairs, 179 concordant hypotensive pairs, and 577 discordant pairs. Both concordant and discordant sib-pairs are being used for analysis of polymorphisms in candidate genes hypothesized to play a role in hypertension.

We selected 5 genes reported to affect blood pressure regulation (11b-hydroxylase, aldosterone synthase, mineralocorticoid receptor) or insulin resistance (CD36, PTP-1b) in both humans and rats. For all genes, the sequence of the human exons and partial intronic sequences were known. We designed primers to amplify the exons and adjacent intronic sequences for each gene from 24 individuals (16 hypertensives and 8 hypotensives from the SAPPHIRe cohort). The resulting amplicons, which were between 400 and 550 bp in size, were subsequently sequenced. All obtained sequences were analyzed by PolyPhred to detect potential single nucleotide polymorphisms (SNPs).

We identified a total of 16 SNPs with a frequency greater than 10%, 10 of which are located in introns. Additional potential SNPs of lower frequency were not investigated further. Only one of the SNPs identified in this screen results in a change in the amino acid sequence. For all genes, at least one SNP was identified.

We have developed allelic discrimination assays (Taqman assays, PE Biosystems) or invader assays (Third Wave Technologies) to facilitate genotyping of the SAPPHIRe cohort. These assays will be used for our ongoing genotyping and association studies.

Program Nr: 2485 from the 1999 ASHG Annual Meeting

A general conditional logistic model for affected relative pair linkage studies. *J.M. Olson.* Epid/Biostat,MetroHlth Med Ctr, Case Western Reserve Univ, Cleveland, OH.

Model-free lod-score methods are often employed to detect linkage between marker loci and complex diseases, using samples of affected sib pairs. Although extentions of the basic one-disease-locus model have been proposed that allow separate inclusion of addition disease loci, covariates, discordant sib pairs, or other types of affected relative pairs, a common framework that can handle simultaneously all of these features has been lacking. I propose a conditional logistic parameterization that generalizes easily to include all of these features. The Risch single-locus affected-sib-pair model is reparameterized in terms of the logarithms of the offspring and monozygotic twin relative risks and written in a form that is generally applicable to all types of affected relative pairs, provided one can compute the relative pair's marker allele-sharing probabilities. Multilocus models are parameterized in terms of the logarithms of joint-allele-sharing-specific relative risks and are easily written in general, multiplicative, and mixed forms. Covariates can be included in both the single- and multilocus models under the assumption that the covariate acts multiplicatively on the relative risks, that is, additively on the log relative risks. Discordant pairs can be added to the model by the addition of a third parameter. Examples using diabetes and simulated data are given to demonstrate uses of the model.

Program Nr: 2486 from the 1999 ASHG Annual Meeting

Genome-wide population genetic mapping of severe bipolar disorder in a Costa Rican isolate. *R. Ophoff[1], M. Escamilla[2], M. Spesny[3], S. Service[1], W. Poon[1], D. Meshi[1], J. Molina[3], R. Ramirez[3], R. Mendez[3], S. Silva[3], M. Ramirez[3], E. Fournier[3], S. Batki[1], C. Matthews[1], T. Neylan[1], E. Roche[1], V. Reus[1], P. Leon[3], L. Sandkuijl[4], N. Freimer[1]*. 1) Dept Psychiatry/Neurogenetics, Univ California, San Francisco, San Francisco, CA; 2) Department of Psychiatry, University of Texas Health Science Center at San Antonio, San Antonio, Texas; 3) Cell and Molecular Biology Research Center and Escuela de Medicina, Universidad de Costa Rica, San Jose, Costa Rica; 4) Department of Human Genetics, Leiden University, Leiden, the Netherlands.

In recent years linkage disequilibrium (LD) analysis has been promoted as a method of mapping disease genes, particularly in isolated populations. So far whole-genome LD strategies have been applied only to simple monogenic traits. Application of such LD strategies to complex traits has awaited development of both sufficiently dense marker maps and powerful statistical methods. We have now completed an initial genome-wide LD screen for a complex trait, namely severe bipolar mood disorder (BP-I). Unrelated affected individuals (n=109) with at least one parent available were collected from the isolated population of the Costa Rican Central Valley. Patients selected for the study had at least 2 hospitalizations, age of onset by age 50 and at least 6 of 8 great-grandparents born in the Central Valley. All individuals, (patients and availble parents, n=288) were genotyped for a set of 1020 microsatellite markers with an average spacing of less than 4 cM. LD was assessed via a two-point method and by an approach that uses haplotypes.

Program Nr: 2487 from the 1999 ASHG Annual Meeting

Mutations and their effect on linkage disequilibrium mapping. *V.S. Pankratz[1], M. Kimmel[2], R. Chakraborty[3]*. 1) Section of Biostatistics, Mayo Clinic, Rochester, MN; 2) Statistics Department, Rice University, Houston, TX; 3) Human Genetics Center, U. Texas School of Public Health, Houston, TX.

The use of linkage disequilibrium to map disease genes in the absence of familial data is of great interest. It provides a framework that can produce refined estimates of the location of disease mutations within a map of marker loci. Its use is especially appealing as disequilibrium can be measured from individuals who are not related. While it is recognized that recombination, drift, mutation and other factors influence genetic associations, many methods of linkage disequilibrium mapping of necessity ignore every component but recombination. However, to obtain good information from population-based data, it is important to utilize methods that account for the genetic characteristics of the population in question. We describe a general approach that allows for the incorporation of mutation processes into two distinct population models that can be used for linkage disequilibrium mapping with one or more marker loci. Using these models, we examine the utility of modeling mutations jointly with recombination. For linkage disequilibrium mapping with a single marker, mutation and recombination events are confounded, as they are indistinguishable in population samples. However, when several marker loci are used jointly to map the position of a disease gene, allowing for mutations provides definite benefits. First, mutations can account for heterogeneity among marker loci. Second, they provide a structure that can describe the addition of new disease genes to the population. Third, mutations serve to smooth multi-point composite likelihoods, improving their properties in genomic regions densely populated by marker loci. Illustrations of these benefits, based on published and simulated data, are provided. (Research supported by grant DMS 9409909 from the NSF, GM 53545 from the NIH, and NLM 1T15LM07093-04 from the National Library of Medicine through the Keck Center for Computational Biology).

Program Nr: 2488 from the 1999 ASHG Annual Meeting

No IL4R alfa gene linkage or association detected in Italian families with atopic asthma by the analysis of 3 mutations. *C. Patuzzo[1], G. Malerba[1], C. Migliaccio[1], E. Trabetti[1], R. Galavotti[1], L. Pescollderungg[2], G. Zanoni[3], A.L. Boner[3], P.F. Pignatti[1]*. 1) Moth-Child,Biology and Genet., Section of Biol. and Genetics,University of Verona, Italy; 2) Division of Pediatrics, Hospital of Bolzano, Italy; 3) Dpt. of Patology, University of Verona, Italy.

As IL4 mediates IgE production by interacting with its receptor, the gene for its receptor is a potential candidate in studies of genetic factors involved in atopic asthma. Two different IL4R alpha gene mutations increase IgE syntesis in vitro and are significatively associated with increased response to some common allergens in a North American population (Q576R, NEJM 1997; 337: 1720), or in a Japanese population (I50V, Nat.Genet.1998; 19: 119). These two mutations, and a third mutation (E375A, B.B.R.C. 1997; 231: 696), were studied in a sample of 851 individuals belonging to 192 Italian families with affected sib-pairs. The following phenotypes were examined: skin prick test (SPT) positivity, total serum IgE, Bronchial Hyperresponsiveness (BHR) to metacholine, and clinical asthma. Atopy was defined as elevated circulating total IgE and/or positive SPT. Non parametric linkage analysis and TDT tests were performed. The following results were obtained. Mutations Q576R and E375A: No allele sharing increase for any phenotype was observed. The frequency of allele 576R was 0.18 (PIC=0.25). The frequency of allele 375A was 0.06 (PIC=0.11). Regression analysis on these two mutations indicated an association with total serum IgE level in sib pairs. Mutation I50V : preliminary data do not show any association with any phenotype.No particular 3 points haplotype was associated with any phenotype.In conclusion, the IL-4Ra gene, in contrast with what reported for other populations, does not seem to be involved in atopic asthma in the Italian population investigated in this study.

Program Nr: 2489 from the 1999 ASHG Annual Meeting

A complete genome screen in sib-pairs affected with the Gilles de la Tourette syndrome. *D. Pauls[1], The Tourette Syndrome Association International Consortium for Genetics[2]*. 1) Child Study Center, Yale University School of Medicine, New Haven, CT; 2) Tourette Syndrome Association Inc., Bayside, Queens, New York, NY.

Gilles de la Tourette syndrome is a neuropsychiatric disorder characterized by waxing and waning multiple motor and phonic tics. Segregation analyses of several family samples have suggested that the mode of inheritance is complex but that genes of major effect appear to be important for the expression of the syndrome. Previous attempts using large multigenerational families to localize susceptibility loci have been unsuccessful. In this report, the results of the first systematic genome scan using 76 affected sib-pair families with a total of 110 sib-pairs will be presented. While no results reached acceptable statistical significance, the multipoint maximum likelihood scores (MLS) for two regions (chromosomes 4q and 8p) were suggestive (MLS > 2.0). Four additional genomic regions, on chromosomes 1, 10, 13 and 19 also gave multipoint MLS scores between 1.0 and 2.0. Subsequent analyses of additional families have yielded supportive evidence for the regions on 8p and 19p. Genotyping is continuing and the most recent results will be presented.

Program Nr: 2490 from the 1999 ASHG Annual Meeting

Localisation of a Gene for Autosomal Recessive Cone-Rod Dystrophy to Chromosome 17p12-13.3. *A.M. Payne[1], S. Khaliq[2], A. Hameed[2], M. Ismail[2], D.A.R. Bessant[1], S.Q. Mehdi[2], S.S. Bhattacharya[1].* 1) Dept Molec Genetics, Inst Ophthalmology, London, England; 2) Research Laboratories, Biomedical and Genetic Engineering Division, Islamabad, Pakistan.

The cone-rod dystrophies (CRDs) are a severe form of inherited retinal dystrophy characterised by the simultaneous involvement of both the cone and rod photoreceptors. CRD can be inherited as an autosomal dominant or recessive, or X-linked trait. Autosomal recessive cone-rod dystrophy (arCRD) is a genetically heterogeneous disease with mutations in the ABCR gene, and many unidentified loci associated with the disease phenotype. In this study linkage analysis was performed on a three generation Pakistani family with arCRD. All the previously reported loci were excluded as the disease region in this family. Significant linkage to the disease gene was obtained with 5 microsatellite markers across a locus situated on chromosome 17p12-13.3. A maximum two point lod score of 3.56 was obtained with marker D17S799 establishing linkage of the disease in our family to this part of chromosome 17p. Recombination events positioned the between D17S938 and D17S921 an estimated genetic distance of 22.3 cM. This new locus (only the second reported for this phenotype) is close to the disease regions of Leber congenital amaurosis, autosomal dominant cone-rod and cone dystrophy and central areolar choroidal dystrophy (RetNet). Ret-GC1 which has been shown to be implicated in Leber congenital amaurosis (Perrault et al., 1998) and autosomal dominant cone-rod dystrophy (Kelsell et al., 1998) and Na K ATPase b 2-subunit, a retinally expressed membrane bound protein responsible for maintaining the K+ and Na+ intracellular concentrations, both map to this region. On screening affected family members for disease causing mutations none were detected in either gene. The disease gene for this novel locus is therefore yet to be identified.

Program Nr: 2491 from the 1999 ASHG Annual Meeting

Automated fragment analysis of capillary electrophoresis data. *M.W. Perlin*[1,2,3]. 1) Cybergenetics, Co., Pittsburgh, PA; 2) School of Computer Science, Carnegie Mellon University, Pittsburgh, PA; 3) Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA.

High-throughput capillary electrophoresis (CE) sequencers such as the ABI/3700 and MD/MegaBACE have become more widely available. These highly parallel CE devices have the potential to greatly increase throughput and reduce labor in genotyping studies that use multiplexed fluorescent microsatellite, SNP, and other genetic markers. However, a key obstacle remains -- the tedious human review of data required for identifying data artifacts. We recently developed a CE processing software module that automatically eliminates certain data artifacts, and have integrated this module into our TrueAllele(TM) automated genotyping software.

The module: (1) Acquires the one dimensional (1D) data of a single capillary for all fluorescent detection channels from the sequencer's data files. (2) Performs initial 1D signal processing (filtering, baseline removal). (3) Automatically classifies the dye colors for a subset of nonprimer peaks, and uses this information to dynamically determine a color separation matrix that is uniquely adapted to the individual capillary run; it separates the dye colors by applying this matrix to the spectrally overlapped data channels in the 1D capillary. (4) Removes the primer region from the data. (5) Tracks the size standard peaks by matching the expected size distribution to the observed peaks. (6) Combines the genetic data traces with the size standard calibration to recast the sampled 1D CE data into a uniformly sized coordinate system. The processed CE traces are then stored for downstream automated genotyping analysis.

At each processing step, the program computes quality measures that can be used to flag problematic data (e.g., low signal, bleedthrough, or incorrect sizing). Interestingly, the third processing step (our new data-adaptive color separation) virtually eliminates the bleedthrough artifact that has complicated multiplexed genetic analysis. Complete data processing for one capillary takes 5-10 seconds on an iMAC computer; the software runs on Macintosh, Windows, and UNIX computers.

Program Nr: 2492 from the 1999 ASHG Annual Meeting

Genetic linkage analysis of prostate cancer families to Xq27-28. *M.A. Peters[1], G.P Jarvik[2], M. Janer[2], L. Chakrabarti[2], S. Kolb[1], E.L. Goode[1,2], M. Gibbs[1], C.C. DuBois[1], E.F. Schuster[2], L. Hood[2], E.A. Ostrander[1], J.L. Stanford[1,2].* 1) Fred Hutchinson Cancer Research Center, Seattle, WA, USA; 2) University of Washington, Seattle, WA, USA.

A family history of prostate cancer has been consistently associated with at least a doubling of risk in first-degree relatives and segregation analysis studies have suggested the existence of an inherited predisposition to prostate cancer. Thus far, four prostate cancer susceptibility loci have been suggested by linkage studies of high-risk prostate cancer families. In the current investigation, we have analyzed families participating in the Prostate Cancer Genetic Research Study (PROGRESS) for linkage to the recently described hereditary prostate cancer locus, HPCX (Nat Genet 20:175-179). PROGRESS is an ongoing Seattle-based study that recruits high-risk prostate cancer families from throughout North America and several other countries.

In this study 129 PROGRESS families have been genotyped at 8 polymorphic markers spanning 15-cM of the HPCX region. These include markers DXS1200 and DXS297, which define the region of the maximum multipoint LOD score as observed in the original HPCX study. Analysis of the total dataset resulted in positive two-point LOD scores at only two markers, DXS984 and DXS1200, with scores of 0.952 at q=0.32 and 0.067 at q=0.38 respectively. In the original HPCX study families without evidence of male-to-male transmission had higher LOD scores at Xq27-28 than those with. Excluding pedigrees with evidence of male-to-male transmission from the PROGRESS families increased the evidence of linkage at DXS1200 (LOD=0.408 at q=0.30), while an additional elimination of families with an unknown mode of transmission resulted in positive LOD scores across a larger portion of the HPCX region. This is consistent with an X-linked locus and locus heterogeneity.

Although, this analysis did not show statistically significant evidence of linkage to the HPCX locus in these 129 PROGRESS families, the results were consistent with a small percentage of the PROGRESS families being linked to this region.

Program Nr: 2493 from the 1999 ASHG Annual Meeting

Power for mapping by admixture linkgage disequilibrium: a case/control simulation study. *C.L Pfaff, M.D Shriver.* Penn State University, University Park, PA.

Admixture between genetically distinct populations creates association between loci. After a few generations, the linkage disequilibrium between unlinked loci decays, and the remaining disequilibrium can be used to map genes (Mapping by Admixture Linkage Disequilibrium). In order to characterize the behavior of MALD, we designed a simulation program that determines the power for MALD depending on a set of parameter values (e.g. number of generations since admixture, proportion of admixture, recombination fraction (q), sample size, and marker and disease allele frequencies). This program examines the power of a case/control study to detect linkage disequilibrium between a marker and a disease allele. Our results show that microsatellite markers are, on average, more powerful than SNPs for MALD. The average power for a SNP marker is 56% (q=0.01, N=300 case, 300 control), whereas an average microsatellite marker achieves >90% power under the same conditions. This difference is mainly due to higher d_C levels (the sum of all positive allele frequency differentials at a locus) at microsatellite loci than at SNP loci. We have found that a high d_C value is the most important characteristic of a good marker for MALD. For example, a marker with a d_C value of 0.71 achieves a power of almost 90% when q=0.06, whereas a marker with a d_C value of 0.38 attains a power of only 57% at the same q. Similarly, markers with high d_C levels demonstrate powers of 89% with only 5% admixture (q=0.001) whereas markers with lower d_C values have powers of 48% under the same conditions. We estimate the average d_C value for microsatellites to be 0.42. Values of d_C above 0.20 generate power estimations >85% at $q \leq 0.01$. Thus, for candidate gene mapping, most microsatellites and many SNPs will provide suitable power. However, for genome-scan mapping a panel of high d markers must be chosen to achieve sufficient power. The results of these simulations indicate that MALD can and should be an important technique for mapping complex disease genes, and has more statistical power than other mapping methods. The results of current efforts comparing the power of MALD to the power of affected sib-pair mapping will also be discussed.

Program Nr: 2494 from the 1999 ASHG Annual Meeting

Role of candidate regions for asthma susceptibility on Sardinian population. *G. Pilia[1,2], N. Olla[1], L. Balaci[1,2], C. Spada[2], M. Fattori[2], M.P. Pinna[2], D. Altea[1,2], G. Sole[1], V. Brancolini[3], M. Devoto[3], A. Cao[1,2].* 1) Istituto di Clinica e Biologia Eta' Evolutiva, Universita' di Cagliari, Cagliari, Italy; 2) Istituto di Ricerca sulle Talassemie ed Anemie Mediterranee, CNR, Cagliari, Italy; 3) Dipartimento di Oncologia, Biologia e Genetica, Universita' di Genova, Genova, Italy.

Asthma is one of the most common chronic diseases in all industrialized countries, affecting 2-4% of the population. Several attempts have already been made to identify genes responsible for asthma susceptibility, but, unfortunately, we are still far from the full characterization of the genes that underlie this complex condition. A problem that is often encountered when dealing with complex traits is the presence of genetic heterogeneity, both of loci and of alleles, which lowers the power to detect linkage. Such a problem is alleviated and may be virtually eliminated when one analyzes an isolated population, in which we expect restricted number of loci and number of mutations to have occurred. Sardinians are one such population. On 100 affected sib-pairs (from 80 Sardinian families with two or more affected sibs) genotyping of 61 markers from the following candidate regions has been performed: Ch.4q33-35, 5q31-q33, 6p21.3, 7p21-15, 11pter-p15, 13q14.1-q14.2 and 16q22.1-q22.1. We carried out an affected sib-pair analysis with the MAPMAKERS/SIBS program. Our results support the view that Ch.4, 5, 11, and 13 may contain genes relevant to asthma. A single region produced suggestive (and nearly significant) evidence of linkage: the region near D13S164 on Ch.13 yielded a single-point lod-score of 3.1. Despite the genetic complexity of asthma, it is therefore possible that our population may provide the resolving power to identify the gene(s) responsible for linkage in each positive candidate regions. This encouraging outcome of our project shows also that the size of the already available Sardinian sample collection is large enough to be used as a primary sample for a whole genome linkage analysis.

Program Nr: 2495 from the 1999 ASHG Annual Meeting

Alpha-2-macroglobulin (Val1000Ile) polymorphism in Finnish late onset Alzheimer's disease patients. *M. Pirskanen[1, 2], M. Hiltunen[1, 2], S. Helisalmi[1, 2], A. Mannermaa[2], A.M. Koivisto[1], M. Lehtovirta[1], I. Alafuzoff[1, 3], H. Soininen[1]*. 1) Department of Neurology, Kuopio University Hospital, Kuopio, Finland; 2) Chromosome and DNA laboratory, Kuopio University Hospital, Kuopio, Finland; 3) Department of Pathology, Kuopio Univ, Kuopio, Finland.

Alpha-2-macroglobulin (a-2M; encoded by the gene A2M) is a serum pan-protease inhibitor that has been implicated in Alzheimer disease (AD) based on its ability to mediate the clearance and degeneration of Ab, the major component of senile plaques characteristic in AD. Recently it was suggested that the A2M polymorphism, Val1000(GTC)/Ile1000(ATC) is associated with the AD. The aim of this study was to find out whether there is an association with A2M and Finnish late onset AD patients. Alpha-2-macroglobulin polymorphism (Val1000Ile) was genotyped from 47 clinically defined late onset AD patients (>65 years) and 50 age matched controls from Eastern-Finland. Genotyping was done with standard PCR and RFLP methods. No association (c2, p = 0.79) was found between AD (A/A 0.33, A/G 0.5, G/G 0.17) and control (A/A 0.28, A/G 0.5 G/G 0.22) genotype. The allele frequencies did not differ statistically (c2, p = 0.56) between groups as the frequencies of A- and G-allele were 0.58 and 0.42 in AD, and 0.53 and 0.47 in controls, respectively. Combining the A/A and A/G or G/G and A/G genotypes to the same category did not reveal differences between AD and control groups (c2, p = 0.62, OR 1.24 (0.49-3.69) and c2, p = 0.67, OR 0.8 (0.33-1.92)). This preliminary data suggests no association between A2M polymorphism (Val1000Ile) and Finnish late onset AD patients.

Program Nr: 2496 from the 1999 ASHG Annual Meeting

Genome-wide screen for the autosomal dominant familial adult myoclonus epilepsy (*fame*) locus. *N.M. Plaster[1], E. Uyama[2], L.J. Ptacek[1]*. 1) Human Genetics, University of Utah, Salt Lake City, UT; 2) Dept. of Neurology, Kumamoto University School of Medicine, Kumamoto, Japan.

Epilepsy is a complex group of disorders affecting approximately 1-3% of the population. The contribution of genetic factors to epilepsy is well recognized. We have collected four familial adult myoclonus epilepsy (FAME) families from Japan, including 22 affected individuals. These individuals' affected status is characterized by autosomal dominant, adult onset, myoclonus of the extremities, and rare generalized tonic-clonic seizures. To determine the location of the *fame* locus, we have performed a genome-wide linkage screen with 65 microsatellite markers spaced 40 to 60 cM apart. The results of linkage analysis indicate four regions of the genome on chromosomes 9, 12, and 13, in which the LOD scores are 0.7 to 1.2. Additional markers flanking these regions are being investigated further, to identify the *fame* locus. Concurrently, we are increasing our marker coverage for the genome-wide screen to a spacing of 20 to 40 cM (>100 markers total), in order to determine additional regions of interest. Identification of the *fame* gene will provide invaluable insights into the etiology of FAME and possibly, phenotypically similar disorders such as juvenile myoclonus epilepsy. Furthermore, investigation of the FAME protein will help us better understand the molecular mechanisms of neuronal hyperexcitability and the etiology of epilepsy.

Program Nr: 2497 from the 1999 ASHG Annual Meeting

High frequency of mutations in the WFS1 (wolframin) gene in patients with Type 1 diabetes. *F. Pociot, Z.M. Larsen, J. Nerup.* Steno Diabetes Ctr, Gentofte, Denmark.

Type 1 diabetes is a multifactorial disease. It has become evident that Type 1 diabetes may be much more heterogeneous than previously assumed. No disease specific mutations have been identified so far. Candidate gene(s) may be derived from knowledge of other diseases where an impaired beta-cell function is observed. Wolfram syndrome is sometimes referred to as DIDMOAD (diabetes insipidus, diabetes mellitus, optic atrophy, and deafness). Insulin-dependent, non-autoimmune diabetes is required for establishing the diagnosis. Pronounced clinical heterogeneity exists in Wolfram syndrome. Therefore, genetic variation(s) leading to only the diabetic phenotype may exist. Recently, the Wolfram syndrome gene was mapped to 4p16. The gene, WFS1 (wolframin), encodes a predicted 890-amino acid transmembrane protein. WFS1 appears to function in survival of islet beta-cells and neurons. Most mutations of WFS1 related to Wolfram syndrome have been located in exon 8. We sequenced exon 8 of 29 Type 1 diabetics, all with age-at-onset less than 10 years, and negative for autoantibodies (GAD65 and IA-2). We have so far identified 5 mutations of the coding part of exon 8, two of which result in amino acid substitutions, and 3 mutations of the 3UTR part of exon 8. In total, we found mutations in 21 of the 29 individuals investigated. Four of the five mutations of the coding region, including one of the missense mutations, were found in at least four non-related individuals, making them potential SNPs useful for large linkage and/or association studies.

Program Nr: 2498 from the 1999 ASHG Annual Meeting

Candidate gene analysis of TGFA, EDN-1, and MTHFR, and the influence of maternal smoking in nonsyndromic cleft lip and palate. *NJ. Prescott, D. Kelberman, MM. Lees, RM. Winter, S. Malcolm.* Molecular and Clinical Genetics, Institute of Child Health, London, UK.

Nonsyndromic cleft lip with or without cleft palate (CL/P) is a common malformation of the face affecting at least 1 in 1000 Caucasian live births. This complex multifactorial disorder exhibits heritability, demonstrated by a high sibling relative risk (l_s»30) where between 2-10 susceptibility loci may have an effect. Factors such as smoking and maternal nutrition are also thought to influence lip and palate formation, interacting with genetic factors to produce the resulting cleft phenotype.

Traditionally, genetic dissection of this complex trait has been via candidate gene selection. We have adopted a genome wide approach for selecting candidate loci. We recently completed a genome search of 91 affected sibpairs and highlighted several areas which deserve further investigation. These include the TGFA locus (p=0.04), previously associated with CL/P, and thought to interact with maternal smoking, 6p24-23 (p=0.008), originally identified through chromosomal breakpoints in cleft individuals, and a third area at 1p36 (p=0.009) which harbours the Methylenetetrahydrofolate Reductase (MTHFR) gene involved in folate metabolism.

For the second phase of this study we have used 72 affected sib pair pedigrees and 48 trios in an ongoing candidate gene evaluation, to examine polymorphic variants of TGFA(2p13), Endothelin-1(6p24-23), and MTHFR(1p36) by T/DT and determine the interaction of maternal smoking. We have demonstrated skewed transmission of the uncommon alleles of TGFA TaqI RFLP, EDN-1 TaqI RFLP and the MTHFR heat labile variant, although sufficient significance has not yet been obtained (p=0.062, p=0.076, p=0.075 respectively),probably due to the limited size of the cohort. Preliminary findings suggest the prevalence of these alleles in cleft offspring do not appear to be significantly associated with maternal smoking. This is an ongoing study and we are continuing to recruit families for T/DT analysis of these and other candidate genes.

Program Nr: 2499 from the 1999 ASHG Annual Meeting

Familial clustering for extreme longevity in humans. *A.A. Puca[1], S.J. Brewster[1], J. Bowen[2], E. Joyce[2], S.B. Ridge[2], M. Shea[2], M. Daly[3], T. Perls[2], L. Kunkel[1].* 1) Genetics Division, Howard Hughes Medical Institute, Children's Hospital and Harvard Medical School, Boston, MA; 2) Gerontology Division, Beth Israel Deaconess Medical Center, Harvard Division on Aging, Boston, MA; 3) Center for Genome Research at the Whitehead Institute for Biomedical Research, Cambridge, MA.

Twin studies of people reaching the age of 85 have previously concluded that genetics play only a minor role in life expectancy. Here we describe 4 families (A-D) which clearly show the segregation of extreme old age.

Family A is composed of 2 males and 4 females aged 100 or greater living in the 17th and 18th centuries. In family B there are 7 individuals, including males, who have lived to at least the age of 101. In family C, you have an sibship of 13 children with 8 reaching extreme old age including a female who is currently 102 years old. The remaining 5 siblings are >80 years of age and in good health. Family D is composed of two branches linked together by a marriage in the 3rd generation. In the 3rd generation, one sees 23/46 individuals living to extreme old age. Of these individuals, 2 lived to the age of 100 or older.

In order to confirm the hypothesis that human longevity is in fact a genetically determined condition, a binomial analysis has been performed for the single most impressive generation of each family. Cohort life tables for the years 1900, 1850 and 1801 were used to estimate the probability of individuals from families A-D to survive to their specified ages. These specific probabilities were then used to calculate a binomial probability of obtaining N individuals achieving their specified ages from a random sample of M individuals belonging to specific birth cohorts. This analysis clearly demonstrates that there is familial aggregation that cannot be explained by random chance but only by the genetic determination of extreme longevity.

Additional families need to be recruited in which there are living individuals of extreme old age in order for linkage analysis to be performed.

Program Nr: 2500 from the 1999 ASHG Annual Meeting

Evidence for the genetic heterogeneity of human papillomavirus-associated epidermodysplasia verruciformis: susceptibility loci on 2p and 17qter. *N. RAMOZ[1], A. TAIEB[2], L.-A. RUEDA[3], M.-J. RUEDA[3], M. FAVRE[1], G. ORTH[1].*
1) Unité mixte PAPILLOMAVIRUS/U190 INSERM, INSTITUT PASTEUR, PARIS, FRANCE; 2) Unité de DERMATOLOGIE PEDIATRIQUE, Hôpital PELLEGRIN-ENFANTS, BORDEAUX, FRANCE; 3) Unidad DERMATOLOGICA, SANTAFE DE BOGOTA, DC COLOMBIA.

Epidermodysplasia verruciformis (EV; MIM#226400) is a rare genodermatosis with an autosomal recessive mode of transmission. The disease is characterized by an abnormal genetic susceptibility to infection with a specific group of related human papillomavirus (HPV) genotypes, including the oncogenic HPV5 associated with the skin carcinomas observed in about half of the patients. EV is thus a model to understand the genetic factors involved in the control of infections with HPVs, in particular the widespread oncogenic HPV genotypes associated with invasive cervical carcinoma. The genes involved in the predisposition to EV HPV infections remain to be identified. By homozygosity mapping in three consanguineous EV families, we recently localized a first EV locus (EV1) to a 1-centiMorgan (cM) interval on chromosome 17qter in a region containing a locus for the susceptibility to familial psoriasis. We had reported that psoriatic patients are likely to constitute a reservoir of HPV5. This suggests that distinct defects of the same gene might be involved in the two skin disorders.

Analysis of microsatellite markers encompassing EV1 locus in two additional consanguineous EV families confirmed the linkage with EV1 in a single family. A genome-wide search in the second family disclosed a new disease locus (EV2) on chromosome 2p. Segregation analysis with microsatellite markers and recombination events allowed to limit the EV2 locus to a 8-cM interval. The disclosure of two distinct susceptibility loci for EV provides evidence for the genetic heterogeneity of the disease and for the multiplicity of controls in infection by oncogenic HPV genotypes.

Program Nr: 2501 from the 1999 ASHG Annual Meeting

Linkage Analysis of Human Systematic Lupus Erythematosus Related Traits. *S. Rao[1], JaneM. Olson[1], C. Gray-McGuire[2], K.L. Moser[2], J.B. Harley[2].* 1) Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH 44109; 2) Arthritis and Immunology Program and Protein Study Program, Oklahoma Medical Research Foundation, Oklahoma City, OK 73004.

Systematic lupus erythematosus (SLE) is an autoimmune disorder characterized by the production of autoantibodies against intracellular antigens, including DNA. Several organ systems may be affected but there is considerable variability among SLE patients in the clinical manifestation of the disease. Little is known about whether observed clinical variability is due to environmental effects, heterogeneity of SLE loci, or modifying genes. We studied 101 SLE affected sib pairs for presence or absence of dermatological (DM), renal (RN), immunological (IM), hematological (HM), neurological (NR), cardiopulmonary (CP) and arthritic (AR) symptoms. Of these, renal and cardiopulmonary showed the strongest evidence of familial aggregation. For multivariate linkage analysis, we obtained nine principal components from the seven traits plus race and age-at-onset. We analyzed genome scan data using the multivariate version of the new Haseman-Elston regression model (SIBPAL2). The largest signal for an individual trait was on chromosome 2 (IM, P=0.00048). The largest multivariate signal was on chromosome 7 (P=0.0029). Of the individual organ systems, DM had the largest effect (P=0.0083) at this location. Because DM is negatively correlated with most other traits, this result might indicate the presence of gene that is responsible for a subtype of SLE or modifying the SLE phenotype.

Program Nr: 2502 from the 1999 ASHG Annual Meeting

Genetic heterogeneity of X-linked thrombocytopenia mapping to Xp11-12. *W. Raskind[1], K. Niakan[1], J. Wolff[1], M. Matsushita[1], T. Vaughan[1], C. Watanabe[2], J. Rios[2], H. Ochs[2].* 1) Dept of Medicine and; 2) Dept of Pediatrics, Univ of Washington, Seattle, WA.

X-linked thrombocytopenia and thalassemia (XLTT, OMIM 314050) is a rare disorder characterized by thrombocytopenia, platelet dysfunction, splenomegaly, reticulocytosis, and unbalanced hemoglobin chain synthesis resembling b-thalassemia trait. In a 4-generation family, we mapped XLTT to chromosome Xp11-12. The maximum lod score possible in this family, 2.39, was obtained for DXS8054 and DXS1003 at q = 0. Recombination events observed for XLTT and DXS8080 and DXS991 define a critical region of 7.65 KcM containing the *WASP* gene responsible for Wiskott-Aldrich Syndrome (WAS, OMIM 301000) and its allelic variant X-linked thrombocytopenia (XLT, OMIM 313900). The manifestations of WAS include thrombocytopenia, eczema and immunodeficiency. In contrast to WAS/XLT in which the platelets are small and bleeding is proportional to the degree of thrombocytopenia, in XLTT platelet morphology is normal and the bleeding time is disproportionately prolonged. To evaluate WASP as a candidate gene for XLTT, cDNA from affected males was examined by ddF. No alterations were seen in the ddF pattern. Western blot and flow cytometric analyses revealed the presence of normal amounts of normal sized WASP protein. Finally, no sequence alteration was found in the 12 WASP exons. X-inactivation patterns were evaluated using methylation differences in the HUMARA locus. As has been reported for WAS and some cases of XLT, almost total inactivation of the XLTT-bearing chromosome was observed in granulocytes and mononuclear cells from one asymptomatic obligate carrier. The only XLTT carrier previously found to have an elevated a:b hemoglobin chain ratio had a skewed pattern favoring activity of the abnormal allele. These findings make it very unlikely that XLTT is another allelic variant of WAS/XLT and strongly suggest that X-linked thrombocytopenia is a genetically heterogeneous disorder. An X-linked syndrome of mental retardation and a-thalassemia (ATR-X, OMIM 300032) is caused by mutations in the helicase gene XNP in Xq13. The possibility that the XLTT gene is a transcription regulator is being investigated.

Program Nr: 2503 from the 1999 ASHG Annual Meeting

Localization of a gene for adult-onset primary open-angle glaucoma to the *GLC1B* locus at chromosome 2cen-q13 in a French-Canadian family. *V. Raymond[1], M. Faucher[1], S. Dubois[1], G. CÔté[2], J.L. Anctil[2], J. Morissette[1]*. 1) Molecular Endocrinology, Laval University Hospital (CHUL) Research Ctr, Quebec, PQ, Canada; 2) Dept. of Ophthalmology, Laval University, Quebec, PQ.

Genetic factors play a major role in the etiology of primary open-angle glaucoma (POAG) and hereditary forms of the disorder have been mapped to at least 7 loci. To counteract this important genetic heterogeneity, we have recruited 24 large French-Canadian glaucoma families showing potential founder effects. One of these kindreds comprised 132 individuals over 4 living generations. To identify the location of the disease-causing gene, we performed a genetic linkage study and an haplotype analysis. Criteria for POAG were: intraocular pressures (IOP) > 22 mm Hg, optic nerve degeneration and open-angle gonioscopy. Out of the 28 persons who were investigated, 7 were diagnosed with autosomal dominant POAG and 2 showed ocular hypertension (OHT) with bilateral IOPs > 22 mm Hg. Age at onset, defined as age at which OHT was first detected, ranged from 38 to 58 years old. Genotyping was performed using Généthon markers selected to cover 9 candidate regions: *GLC1A* at 1q23-q25, *GLC1B* at 2cen-q13, *GLC1C* at 3q21-q24, *GLC1D* at 8q23, *GLC1E* at 10p14-p15, *GLC1F* at 7q35, *IRID1* at 6p25, *IRID2* at 4q25 and 11p13. For linkage analyses, OHT and POAG patients were considered affected. Evidence of linkage was observed between the affected phenotype and markers located at *GLC1B*. Two-point lod score values reached a maximum of 2.97 with marker AFMa333vh5 (D2S388) at q=0. Preliminary multipoint analyses supported these data with lod score values near 3. The same characteristic haplotype comprising 12 markers was recognized in all persons affected by either POAG or OHT. Two key affected recombinants confined the disease region within an interval of less than 8 cM. These findings demonstrated that the *GLC1B* locus is associated with adult-onset POAG in at least one French-Canadian family. These observations also confirmed the mapping of a glaucoma locus at 2cen-q13 in families ascertained in the United Kingdom (Stoilova & al., Genomics 1996, 36:142-150).

Program Nr: 2504 from the 1999 ASHG Annual Meeting

Association of a CD14 promoter polymorphism and atopic phenotypes. *N.E. Reijmerink[1], G.H. Koppelman[1,2], O.C. Stine[3], D.G. Wiesch[3], D.A. Meyers[3], E.R. Bleecker[3], D.S. Postma[1]*. 1) University Hospital Groningen,; 2) Beatrixoord Rehabilitation Center, The Netherlands; 3) Center for the Genetics of Asthma and Complex Diseases, University of Maryland, Baltimore, USA.

Background: Asthma, a serious chronic airway disease characterized by bronchial hyperresponsiveness and airway inflammation is a complex disease with major a genetic component. Atopy, an immunologic state of hypersensitivity to environmental allergens characterized by high levels of IgE is a very important aspect in asthma. A recent study suggests that a polymorphism in the promoter region of CD14 may have a central role in regulating atopic phenotypes, such as high levels of IgE and positive skintest responsiveness. CD14 is a multifunctional surface receptor, localized on monocytes and macrophages. One of its functions is to bind lipopolysaccharide (LPS). After binding to LPS, CD14 may inhibit IgE production.

Methods:A case-controle study was used and phenotypes related to atopy as well as asthma were studied.159 adult patients with asthma and 158 spouses (n=317) from a restricted Dutch population were analyzed with restriction enzyme *AVA*II, for a polymorphism in the promoter region of the CD14 gene (a C to T transition at position -159).

Results: Allele frequencies for CC were 25.9%, CT 50.8% and TT 23.3%. In a recessive model, CC homozygotes had higher levels of serum total IgE (p=0.036) and higher numbers of positive skintests (p=0.008). There was no significant association between this polymorphism and asthmatic phenotypes.

Conclusion: This study suggests that the CD14 gene plays an important role in regulating atopic phenotypes.

We thank Dr.F.Martinez for the use of the primers. Supported by the Netherlands Asthma Foundation, the Jan Kornelis de Cock-Stichting and the National Institute of Health(HL48341).

Program Nr: 2505 from the 1999 ASHG Annual Meeting

Modification of a YAC deletion vector aids SNP detection and mapping. *J.H Riley, K. Lewis, R. Khakhar, L. Hosking, I.J. Purvis.* Molecular Genetics, Glaxo Wellcome Medicines Research Centre, Stevenage, Hertfordshire, U.K.

A yeast artificial chromosome (YAC) deletion method has been developed to aid the isolation and mapping of single nucleotide polymorphisms (SNPs) across two complex disease loci. The method exploits recombination in the yeast cell between the linearised YAC deletion vector, pBCL and the human insert of the YAC. The recombination is directed via human alu repeats which undergo random recombination and subsequent deletion of the YAC to generate sets of deletion derivatives from the parental YAC. Ends of deletants can be captured, sequenced and SNPs subsequently identified and physically mapped. Initial experimentation generated deletion derivatives containing large gaps between the deletants of up to 500kb and very few deleted YACs of less than 150kb. To improve the coverage and hence the mapping, modifications to the vector were made. A vector containing an inverted alu was constructed and a further four vectors containing different alu family members were created. The experiments were designed to enable the specificity of the recombination event to be determined and to generate a nested set of deletants with a largest gap size of no greater than 250kb and an average gap size of 100kb. This technology was applied in two complex disease loci mapping to different chromosomes. YAC contigs were generated for each. Across both loci YACs were subjected to the deletion protocol using pBCL alone. The recombination for one YAC clearly showed a large number of deletants of the same size. However, when the deletion experiment was repeated with the newly constructed vectors, a range of different sized deletion derivatives was observed. This demonstrates the specificity of the recombination event and the advantage of utilising more than one vector. The YAC deletion technology not only provides a tool for accurate mapping of SNPs but it also enables sequences to be generated at specific points across a locus by end capture of the deletion derivatives.

Program Nr: 2506 from the 1999 ASHG Annual Meeting

DataTracker: Comprehensive software for data quality control protocols in complex disease studies. *J.B. Rimmler, C.S. Haynes, J.G. McDowell, J.E. Stajich, C.S. Adams, B.D. Slotterbeck, A.R. Rogala, S.G. West, J.R. Gilbert, E.R. Hauser, J.M. Vance, M.A. Pericak-Vance.* Center For Human Genetics, Duke University Medical Center, Durham, NC.

The study of human disease traits of complex genetic architecture is a rapidly expanding area. High throughput genotyping is now the norm. The structure of families under analysis is smaller and more variable and sample sizes are increasing. Protocols dealing with quality control [QC] issues of these data, however, have lagged behind technological advancement. Thus, we have designed and implemented a series of protocols for each of the critical steps in the processing of the associated laboratory data. In addition we have developed a program, DATATRACKER, to perform the quality control [QC] assessments of these data. Specifically, an auxillary program, ScreenKey_Create, uses DNA sample lists to automatically generate gel plate loading diagrams, gel reader template files, and QC sample keys. QC Samples (or duplicated samples) are positioned on a gel [6 per gel] and DNA is dispensed robotically for analysis. Technicians are blinded with respect to the location of the QC duplicates to avoid bias in interpretation of results. These steps serve to ensure the consistency of readings and to identify problems such as misloads, gel reading errors, and sample mix-ups across gels.

Once the initial genotype generation is complete, the results undergo a series of QC checks that identify problems such as incorrect formatting, typographical errors, inconsistancy of CEPH standards, and low efficiency levels. Problems are automatically e-mailed to the technician and QC supervisor for re-examination and correction. QC summary statistics are databased by projects and markers, and summary reports automatically produced. Statistics databased include the genotyping list used, date of QC analysis, marker efficiency, % QC match, technician, and date data passed all QC checks. Specific protocols, report examples and summary statistics will be presented for this system as a prototype for other researchers involved in the study of complex traits.

Program Nr: 2507 from the 1999 ASHG Annual Meeting

Familial congenital isolated ptosis with dominant inheritance and equal expression in males and females: a new truly dominant X linked condition. *D.O. Robinson[1], A.G. Tyers[2], T.F.W. McMullan[1,2]*. 1) Wessex Regional Genetics Lab, Salisbury District Hosp, Wiltshire, England; 2) Dept. of Ophthalmology, Salisbury District Hosp., Wiltshire, England.

Ptosis (blepharoptosis) is defined as the abnormal drooping of the upper eyelid and results in reduction of the field of vision. The condition is a feature of many syndromes and can be both unilateral or bilateral, congenital or acquired. Ptosis can also be present as a sole feature with no other abnormalities. We have studied a family from Wessex in the UK in which dominantly inherited congenital ptosis is segregating. Ptosis is present as a sole feature, is bilateral and equal in severity in males and females. DNA samples from 28 family members including 15 affected individuals have been analysed by linkage analysis. No male to male inheritance was evident so analysis was carried out using markers along the whole length of the X chromosome at 10-15cM intervals. Linkage to Xq25-27.1 was established with a LOD score of 2.7 at DXS1212 with no recombination. Two females in the pedigree have the at risk haplotype but do not have ptosis. This could be either due to double recombination or to non-penetrance. In one of these females unilateral X inactivation was shown in DNA from blood which could be the cause of non-expression of the phenotype. The only other documented pedigree with ptosis as the sole feature and with genetic linkage data is that of Engle et al (Am J Hum Genet 1997, 60, 1150-1157). They established linkage to chromosome 1p32-34.1 in an American family with dominant inheritance. We have therefore identified a second gene for isolated ptosis and defined a new condition namely X-linked dominant congenital isolated ptosis. Furthermore there appears to be equal expression of ptosis in both males and females which is very unusual. For most X linked dominant conditions males are affected more severely than females. This therefore defines a truly dominant X linked condition.

Program Nr: 2508 from the 1999 ASHG Annual Meeting

Autosomal dominant pure Spastic Paraplegia in a Brazilian family: Linkage to chromosome 8q and study of muscle syntrophin b**1.** *P.S. Rocco[1], M. Vainzof[1,2], S.C. Froehner[3], S.K.N. Marie[2], L.M. Kunkel[4], M.R. Passos-Bueno[1], M. Zatz[1]*. 1) Centro de Estudos do Genoma Humano, Dept. of Biology, IB-USP, Sao Paulo, Brazil; 2) Dept. of Neurology, FMUSP; 3) Dept. of Cell and Molecular Physiology, School of Medicine, UNC-Chapel Hill, PH, USA; 4) Division of Genetics and the Howard Hughes Medical Institute, Childrens Hospital, Boston, USA.

Autosomal Dominant hereditary spastic paraplegia (AD-HSP) include a genetically heterogeneous group of degenerative disorders of the central motor system, characterized by progressive spasticity of the lower limbs. AD-HSP may be sub classified into pure and complicated forms based on the presence of additional neurological features. Four loci for pure AD-HSP have been identified up to date: SPG3 on chromosome 14q, SPG4 on chromosome 2p, SPG6 on chromosome 15q, and more recently SPG8 on chromosome 8q (Hedera et al., 1999). We have analyzed a Brazilian family with 15 affected individuals by pure AD-HSP (8 males and 7 females) who developed insidiously progressive gait disturbance with onset at age 18-26 years. Linkage analysis performed with 13 family members (6 affected and 7 normal) excluded SPG3, SPG4 and SPG6 as candidate regions. However, positive LOD scores were obtained with markers close to the SPG8 locus (Z max=3.01 at theta=0 for D8S1804, D8S568, and MYC). In this region lies syntrophin b1 gene (SNT2B1), a widely expressed dystrophin-associated protein and therefore a good positional and functional candidate for this disease. We performed immunohistochemical (IF) and Western Blot (WB) studies using an antibody to syntrophin b1 in a muscle biopsy from one affected male. The distribution, expression and MW of the protein were comparable to normal control. Therefore, it is unlikely that defects in this protein is causing SPG8, at least in the present family. Additional DNA studies will be performed to definitively exclude the SNT2B1 gene as a candidate for SPG8. We are also currently performing linkage analysis with other markers and including other 4 affected individuals in an attempt to narrow down the previous mapped region. Supported by FAPESP, CNPq, PRONEX and CAPES.

Program Nr: 2509 from the 1999 ASHG Annual Meeting

Localization of genes predisposing to acute leukemia in Down syndrome. *P.K. Rogan[1], D.W Sabol[2], P. Close[3].* 1) Section of Medical Genetics & Molecular Medicine, The Children's Mercy Hospital and Clinics, Kansas City, MO; 2) Phylogenetix Laboratories, Pittsburgh, PA; 3) Division of Pediatric Hematology/Oncology, Harbor-UCLA Medical Center, Torrance, CA.

Constitutional chromosome 21 aneuploidy in blasts from children with Down syndrome can predispose to additional segregation errors resulting in either increased gene dosage or loss of heterozygosity (LOH). Eight of 16 patients enrolled in protocol CCG-B957 exhibited reduction to germline homozygosity at one or more loci on the non-disjoined chromosome. Three genetic intervals displayed disomic homozygosity and significant linkage to the disease locus in a cohort of patients with acute lymphocytic leukemia (ALL; n=5), and acute myeloid leukemia (AML; n=3). Linkage to centromeric 21q loci (Z_{max}=2.41)confirmed and refined the interval associated with transient leukemoid reaction and AML-type M7 in this population. LOH at D21S16 and D21S13 on the normally disjoined chromosome (a possible second hit) was also detected in 2 individuals who exhibited disomic homozygosity within this interval. The second interval (Z_{max}=3.30) overlapped the region containing a familial platelet disorder disease locus (FPDMM). The third interval spanned HMG14 through D21S266 (Z_{max}=3.01). Somatic LOH was also detected in 2 patients at D21S1434 and D21S1436, respectively, and in 3 patients at D21S1280 and D21S262, both of which are linked to the TIAM1 gene. Patients may carry homozygous recessive alleles on non-disjoined chromosomes that are sometimes unmasked by somatic mutations on the normally disjoined chromosome.

Program Nr: 2510 from the 1999 ASHG Annual Meeting

FRAGMENT ANALYSIS PERFORMANCE ON THE ABI PRISM® 3700 DNA ANALYZER. *M. Roque-Biewer, Y. Wang, K. Rogers, A. Wheaton, M. Rivera, N. Caffo, P. Dong.* Genetic Analysis, PE Biosystems, Foster City, CA.

The 3700 DNA Analyzer is a fully automated high-throughput capillary array instrument designed to operate in a 24-hr unattended mode that can process hundreds of samples daily. In fragment analysis, the current dye set uses 6FAM, HEX, NED and ROX. The use of 4 dyes in a single lane for fragment analysis allows the generation of up to 400 genotypes per day on the ABI PRISM 310 Genetic Analyzer and 4,500 genotypes per day on the ABI PRISM 377 DNA Sequencer (96-lanes/3runs per day). The new 3700 DNA Analyzer, will be able to generate up to 15,000 genotypes in a 24-hr period. We have optimized the 3700 DNA Analyzer for microsatellite applications. We will show resolution and sizing precision across the capillary array under standard and varying conditions (run voltage, injection time, injection voltage, etc.). Utilizing the associated GeneScan NT and Genotyper NT software, we will show that the system can assign a size in nucleotide (nt) to a fragment with standard deviation of < 0.15 nt up to 300 nt. We will also show compatibility of the linkage mapping suite of reagents with the instrument.

Program Nr: 2511 from the 1999 ASHG Annual Meeting

Linkage of a Large Family with Hereditary Spastic Paraplegia to Chromosome 2p. *D.R. Rosen[1], N.H. Chapman[2], C. He[1], T.F. Thurmon[3], E.M. Wijsman[2, 4].* 1) Div Genetic Disorders, Wadsworth Ctr, Albany, NY; 2) Dept. of Biostatistics, University of Washington Seattle, WA; 3) Louisiana State University Medical Center, Shreveport, LA; 4) Division of Medical Genetics, University of Washington Seattle, WA.

Hereditary spastic paraplegia (HSP) is a group of disorders caused by the degeneration of corticospinal motor neurons, resulting in loss of strength and coordination of the legs. We have performed linkage analysis on a large American family with uncomplicated hereditary spastic paraplegia. Clinical features of this family's HSP include gait disturbances and hyperreflexia; the family is remarkable in that leg spasticity and a positive Babinski sign are not obligate features of the disorder. The mode of inheritance in this family is autosomal dominant. Current size of this family is greater than 600 individuals covering 9 generations. Of these, 49 cases of HSP have been documented. Blood samples from 38 family members, including 13 HSP-affected individuals, were collected. Linkage analysis was performed on a total of 83 family members, including this set of 38 individuals, for four previously reported HSP loci at chromosomes 2p, 8q, 14q, and 15q. Negative lod scores were obtained for 2 markers at 8q, 3 markers at 14q, and 3 markers at 15q. Positive lod scores were obtained for 13 DNA markers on chromosome 2p. A peak lod score of 3.87 at a recombination fraction of 0.001 was obtained for D2S2374. These results suggest that this family has a mutation linked to the SPG4 locus on chromosome 2. Because the HSP in this family was previously demonstrated to exhibit anticipation, further evidence for a dynamic mutation at the SPG4 locus is suggested.

Program Nr: 2512 from the 1999 ASHG Annual Meeting

Genetic Mapping of a Locus for Amish Microcephaly. *M. Rosenberg[1], R. Agarwala[4], M.S. Hilliard[1], J. Weber[5], D.H. Morton[3], A.A. Schaffer[4], R.I.. Kelley[2], L.G. Biesecker[1].* 1) GDRB, NHGRI/NIH, Bethesda, MD; 2) Kennedy-Krieger Inst, Baltimore, MD; 3) The Clinic for Special Children, Strasbourg, PA; 4) NCBI, NLM, NIH, Bethesda, MD; 5) Marshfield Clinic, WI.

Amish microcephaly is a syndrome that includes severe congenital microcephaly, hypertonia, irritability, and premature death, usually at six to 15 months of age. The disorder is inherited in an autosomal recessive pattern and is only known to occur in Old Order Amish who have ancestors in Lancaster County, Pennsylvania. We analyzed an extended kindred with this condition that included 21 nuclear families who had an affected child. A single pedigree was constructed that connected all of these nuclear families in a systematic and automatic manner using PedHunter software, the Steiner tree software package of T. Kock and A. Martin and the Amish Genealogy Database. A whole genome linkage scan was performed on a subset of eight families using semi-automated fluorescent genotyping. Two-point LOD scores were calculated for all markers and the phenotype using a model of autosomal recessive inheritance and full penetrance with the FASTLINK software package 4.0P. Positive markers with a LOD > 3 or (LOD > 2 and an adjacent locus with LOD > 1) were further analyzed by typing additional flanking markers and additional samples. Ten markers met these criteria and additional manual genotyping excluded all but one of these loci. The remaining positive screening marker was D17S1301. We mapped the disease locus to chromosome 17q with a three-point LOD score of 3.87 using markers D17S1603 and D17S1301 and a pedigree with six consanguinity loops. We conclude that Amish microcephaly is a single gene disorder that perturbs prenatal CNS development and the gene for this disorder maps to distal chromosome 17q.

Program Nr: 2513 from the 1999 ASHG Annual Meeting

Candidate regions in MS monitored using SNP microarray. *J. Saarela[1], H.M Frostad Riise[1], T. Pastinen[2], M. Schoenberg Fejzo[1], D. Chen[1], S. Kuokkanen[3], L. Peltonen[1].* 1) Human Genetics, UCLA, school of medicine, Los Angeles, CA; 2) University of Helsinki and National Public Health Institute, Dept. of Molecular Genetics, Helsinki, Finland; 3) Cancer Genetic Branch, NHGRI, NIH, Bethesda, MD, 20892.

Multiple sclerosis (MS) is a demyelinating disorder of the central nervous system with a putative autoimmune etiology. It is the most common cause of acquired neurological dysfunction of young adults. Genetic susceptibility to MS is implicated on the basis of twin and family studies. Several putative susceptibility loci have been identified by other groups and us but only the HLA region on chromosome 6p has repeatedly shown definitive association to MS. Among the interesting, putative loci are two relatively wide chromosomal regions on chromosomes 5p14-p12 and 17q22-q24, which are syntenic to mouse chromosomes 15 and 11, respectively, and harbor susceptibility loci for murine EAE. To restrict these chromosomal areas by identifying a shared haplotype between affected individuals, we produced a microarray for genotyping SNPs spanning these regions. In the first SNP microarray we used 22 SNPs on chromosome 5 and 17 on chromosome 17 from existing databases. The second SNP array contains novel, intragenic SNPs created from ESTs mapped to these regions. We have also sequenced several candidate genes on 17q22-q24 including ICAM-2, PECAM 1, myeloperoxidase, and apolipoprotein H and identified several novel polymorphisms. Our SNP array utilizes specificity of the RNA reverse transcriptase to detect the variable nucleotide. PCR products from genomic DNA containing either T3 or T7 RNA polymerase transcription start sites, are *in vitro* transcribed to RNA, and subsequently converted to cDNA with the RT enzyme and one of the detection primers attached to the slide having the right variable nucleotide as the most 3' nucleotide. The RT enzyme incorporates CYP-labeled nucleotides to cDNAs which are detected by a laser scanner. In the Finnish MS study material we will monitor for association between MS and SNPs on the linked chromosomal regions. This approach should aid us in identification of predisposing genes for MS.

Program Nr: 2514 from the 1999 ASHG Annual Meeting

An Updated Genome Screen for Platelet Monoamine Oxidase (MAO) Activity. *N.L. Saccone[1], J.P. Rice[1], N. Rochberg[1], J.T. Williams[2], A. Goate[1], T. Reich[1], S. Shears[1], W. Wu[1], J.I. Nurnberger Jr.[3], T. Foroud[3], H.J. Edenberg[3], T.-K. Li[3]*. 1) Washington University School of Medicine, St Louis, MO; 2) Southwest Foundation for Biomedical Research, San Antonio, TX; 3) Indiana University School of Medicine, Indianapolis, IN.

We have performed new linkage analyses for platelet MAO-B activity on three samples: a previously analyzed sample (wave 1, 95 extended families containing 827 phenotyped members), a distinct sample (wave 2, 120 extended families containing 797 phenotyped members), and the combined sample. These families were recruited for the Collaborative Study on the Genetics of Alcoholism (COGA). Sibpair-based analyses were performed using MAPMAKER/SIBS; variance component analyses of extended pedigrees were performed using SOLAR. Platelet MAO-B activities were measured using a tryptamine assay, and values were corrected according to subjects' cigarette smoking status, gender, and center where blood was drawn.

Variance component analysis gave lod scores of 1.5, 2.3 and 3.2 in the respective samples in a region of chromosome 9 near D9S261; the lod score in the combined data was 1.4 greater than the next highest in the genome. Sib pair analyses of chromosome 9 with independent pairs gave respective lod scores of 0.8, 1.1 and 1.6, which in the combined data was the second largest signal in the genome via this method.

Haseman-Elston based regression using independent sib pairs found suggestive linkage on chromosome 2 (lod=2.0, 2.5 and 2.8 in the respective samples); variance component analysis gave lod scores of 0.8, 0.5 and 1.3, respectively, in this region.

The consistency across datasets and methods for the chromosome 9 finding is exciting. Variance component analysis supports a potential linkage of MAO activity to chromosome 9 with a lod over 3 in the combined sample; this finding may reflect the information gained by jointly considering all pedigree relationships. Further analyses are underway to understand the differences in results obtained by the two methods on chromosome 2.

Program Nr: 2515 from the 1999 ASHG Annual Meeting

A novel locus DFNA23 for prelingual autosomal dominant hearing loss maps to 14q in a Swiss German kindred.

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A novel locus for non-syndromic hearing loss has been identified in a Swiss German kindred with autosomal dominant bilateral sensorineural hearing loss. A total of 25 members of this family over three generations were ascertained for study. Eleven of the family members were considered affected, 13 family members unaffected and one family member was excluded from analysis due to uncertain affection status. No evidence for acquired risk factors predisposing to hearing loss was observed for any of the affected individuals. The history and records of the patients suggested a prelingual onset (at birth or in the first years of life). The majority of the affected family members did not display progression of hearing loss during a follow up period which varied between 6 and 32 years. In only two cases the hearing loss showed progression. Audiometry displayed a bilateral and symmetrical hearing loss. In 6 cases there existed a conductive component (which produced an air-bone-gap between 10 dB and 40 dB). All audiograms showed sloping curves with normal hearing ability to mild hearing loss in low frequencies, normal to profound hearing loss in mid frequencies and moderate to profound hearing loss in high frequencies.

A genome scan was carried out in this family and a maximum multipoint los score of 3.9 was obtained. The locus which segregates in this family maps to 14q and does not overlap with DFNA9. Additional work is being carried out to further refine the genetic region for this novel locus.

This work was supported by the NHLBI Mammalian genotyping service, NIH-NIDCD grant DC03594 and the American Hearing Research Foundation. .

Program Nr: 2516 from the 1999 ASHG Annual Meeting

Two regions of linkage on chromosome 8 in Type 1 diabetes. *M.M. Sale[1], J.C. Charlesworth[2], C.C. Simpson[1], G. Morahan[3], J. Pendleton[4], D.W. Bowden[4].* 1) Menzies Centre for Population Health Research, University of Tasmania, Hobart, Australia; 2) Dept Biochemistry, University of Tasmania, Hobart, Australia; 3) The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia; 4) Wake Forest University School of Medicine, Winston-Salem NC.

While several Type 1 diabetes susceptibility loci besides HLA and the insulin gene have been reported, other loci remain to be identified to account for the familial clustering observed. We have identified two regions suggestive of linkage on chromosome 8 in a U.S. Type 1 diabetes population of 137 affected sibling pairs derived from 121 families. Using multipoint analysis, the first region of linkage centred on D8S281 produced a non-parametric LOD score (NPL) of 3.0 (p=0.001). When combined with 45 Australian families, the NPL rose to 3.3 (p=0.0005). Conditioning on HLA and sex indicated a bias to HLA-discordant sibs and families with predominantly male offspring in both American and Australian populations. Linkage was not observed in 140 British IDDM families. Located approximately 20cM proximal to this peak, a second region of linkage was observed at D8S271 with a NPL of 3.1 (p=0.0008) in the U.S. families alone. Linkage at this locus was not detected in the British population, and was equivocal in the Australian families. Significant results using the transmission disequilibrium test (p=0.024 and 0.002) were obtained with alleles at the two peak loci. A positional cloning approach will be used to localise and identify two possible Type 1 diabetes susceptibility genes.

Program Nr: 2517 from the 1999 ASHG Annual Meeting

Linkage to the Lipoprotein Lipase Gene in an Extended Utah Kindred with Hypoalphalipoproteinemia: The -93G/D9N variant predisposes to low HDL. *M. Samuels[1], K. Forbey[1], A. Bandley[1], K. Bulka[1], B. Wardell[1], J. Reid[1], P. Hopkins[2], S. Hunt[2], D. Ballinger[1], M. Skolnick[1], S. Wagner[1]*. 1) Myriad Genetics, Salt Lake City, UT; 2) Department of Cardiovascular Genetics, University of Utah, Salt Lake City, UT.

Defects in the lipoprotein lipase (LPL) gene have been associated with dyslipidemia in the general population. Several rare mutations in the gene, as well as two common coding region polymorphisms, D9N and N291S, exhibit deleterious effects on circulating lipid levels. Such mutations have generally been discovered by random screening of probands. Using a linkage-based approach, we have identified a large Utah kindred segregating the D9N variant. The variant shows significant causal evidence for dyslipidemia in the kindred by multipoint linkage analysis. The variant shows the most penetrance for an hypoalphalipoproteinemia phenotype, but is also associated with hypertriglyceridemia. This is the first example using genetic linkage analysis to identify a specific mutation causing predisposition for dyslipidemia.

Program Nr: 2518 from the 1999 ASHG Annual Meeting

A genome-wide search for genes predisposing to familial psoriasis using a stratification approach. *L. Samuelsson[1], F. Enlund[1], A. Torinsson[1], A. Inerot[2], C. Enerback[2], J. Wahlstrom[1], G. Swanbeck[2], T. Martinsson[1]*. 1) Dept of Clinical Genetics, Univ Gothenburg, Sahlgrenska University Hospital/East, Gothenburg, Sweden ÅÅ; 2) Dept of Dermatology and Venereology, Univ Gothenburg, Sahlgrenska University Hospital, Gothenburg, Sweden.

In the search for psoriasis susceptibility loci, we have performed a genome scan using microsatellite markers spaced by 10 cM and a family set of 134 affected sibling pairs. Genotyping results were analyzed for non-random excessive allele-sharing between sib pairs, using GENEHUNTER ver 1.1. A stratification approach was applied to increase the homogeneity of the material by use of an operational definition of joint complaints among affected individuals. Significant linkage to the HLA-region on chromosome 6p in a cohort including 42 families without joint complaints (NPL-value of 2.83, p=0.002) supports the validity of this operational definition as it replicates results from earlier linkage reports using similar stratification criteria. New candidate regions on chromosome 3 and 15 were identified. The highest NPL-values in this study, 2.96 (p=0.0017) and 2.89 (p=0.0020), were reached on chromosome 15 in a subgroup with joint complaints and on chromosome 3 in a subgroup without joint complaints. In addition, confirmation of previously reported loci was established on chromosomes 6p, 14q, 17q and 20p. This study indicates that distinct disease loci might be involved in psoriasis etiology for various phenotypes.

Program Nr: 2519 from the 1999 ASHG Annual Meeting

Robust regression methods for complex traits: Simultaneous linkage and linkage disequilibrium. *D.J. Schaid, C.M. Rowland.* Section Biostatistics, Mayo Clinic, Rochester, MN.

Robust methods to evaluate genetic linkage of markers with complex traits typically do not allow for the possibility of linkage disequilibrium (*LD*), which can result in a loss of power if *LD* exists. The transmission/disequilibrium test (*TDT*) takes advantage of both linkage and *LD*, and provides a valid test for linkage in the presence of *LD*. However, the *TDT* has several limitations: typically, it is applied to only affected offspring, whereas more complex traits may be relevant, such as ordinal or continuous traits; it does not allow for multiple siblings when testing for *LD* in the presence of linkage; and it does not allow for environmental covariates.

To take advantage of both linkage and *LD*, we propose a new regression method that uses marker allele sharing between sibships, to capture *LD* and use it to impute parental linkage phase, and allele sharing within sibships, to account for linkage. The proposed method extends the binomial mixture likelihood for linkage by (1) incorporation of *LD* with an arbitrary number of marker alleles via regression parameters that account for between-family sharing of marker alleles, and hence incorporates different parental phase probabilities, and (2) having the probability of transmission of parental marker alleles depend on the offsprings' traits. This general regression method allows for simple traits (affected/unaffected) as well as more complex traits (continuous; multiple traits) and covariates.

Our proposed method evaluates the transmission of alleles from each heterozygous parent to their offspring, irregardless of whether parents are from the same family. The advantage of considering parents separately is a gain in robustness, without need to consider mode of inheritance, but requires pseudo-likelihood methods to account for dependencies of allele transmission between parents of the same offspring. This approach, as well as a method that considers both parents jointly, will be presented, as well as simulations demonstrating the statistical properties of these methods.

Program Nr: 2520 from the 1999 ASHG Annual Meeting

Fine mapping asthma- and atopy-susceptibility loci on chromosome 5q23-31 in the Hutterites. *D. Schneider[1], A. Tselenko[1], J. Andal[1], D.M. Hoki[1], R. Daniel[1], K. True[1], E.L. Sorensen[1], S. Willadsen[1], N.J. Cox[1,2], C. Ober[1]*. 1) Department of Human Genetics, University of Chicago, Chicago, IL; 2) Department of Medicine, University of Chicago, Chicago, IL.

The cytokine gene cluster on 5q23-31 includes a plethora of loci that are functional candidates for asthma and atopy susceptibility alleles. Thus, it is not surprising that many candidate gene and linkage studies of asthma and atopy have identified this region as housing genes that influence these phenotypes. We previously reported linkage to this region in the Hutterites, a founder population of European ancestry. D5S1480 showed evidence of linkage to asthma by both the likelihood ratio test (LR) and the TDT (P = 0.0079and 0.0091, respectively) (Ober et al., 1998, *Hum Molec Genet* 7:1393) and D5S642 showed evidence of linkage to atopy by the TDT (P = 0.0082) (Ober et al., 1999, *Clin Exp Allergy*, in press). To identify the 5q-linked asthma and atopy susceptibility loci, we genotyped a sample of 750 Hutterites for 24 markers spanning 30 cM (average spacing 1.25 cM), including one or more STRPs or SNPs in the following candidate genes: *IL4*, *IL9*, *CD14*, *B2ADR*, and *CSF1R*. A region of <2 cM and including D5S1480 shows the strongest evidence of linkage with asthma. None of the candidate genes examined account for our evidence of linkage to asthma in this region, suggesting that an as yet unidentified gene on 5q31 confers susceptibility to asthma. Furthermore, a haplotype comprised of alleles at D5S1480 and D5S210 is associated with asthma (P = 0.019), identifying a haplotype that likely carries the 5q-linked asthma susceptibility allele in the Hutterites. The candidate gene, CD14, showed significant association with atopy, measured by positive skin prick test (+SPT) to ³1 of 14 allergens (P = 0.0009), suggesting that this gene may be the atopy-susceptibility locus on 5q in the Hutterites. (Supported by HL49596 and HL56399).

Program Nr: 2521 from the 1999 ASHG Annual Meeting

Integrated map of chromosome 17q critical region in multiple sclerosis. *M. Schoenberg-Fejzo[1], J. Saarela[1], D. Chen[1], M. Parkkonen[1], S. Kuokkanen[2], A. Palotie[1], L. Peltonen[1].* 1) Department of Human Genetics, Univ California, Los Angeles, CA 90095; 2) Cancer Genetics Branch, NHGRI, NIH, Bethesda, MD, 20892.

Multiple sclerosis (MS) is a neurological disease characterized by inflammatory demyelination of the CNS with a typical onset in the second to the fourth decades of life. In the past, multiple studies have revealed evidence for a genetic component to MS and genome-wide screens have identified several suggestive chromosomal regions. Of particular interest, studies of Finnish and British MS-families singled out chromosome 17q22-q24 as an area for further study. We have screened 32 additional markers mapping to 17q22-q24 in the original Finnish MS-families. D17S1825 has a maximum two point LOD score of 3.42. All other markers in the region of about 20 cM also generated positive lod scores. Multipoint analysis with 23 ordered markers using Genehunter and Simwalk2 would position the putative MS locus within a 5 cM region flanked by markers D17S807 and CHLC.GATA108A5. An integrated map over the 17q critical region in MS has been created using 60 markers covering an approximately 15 Mb/10 cM region. The map was built using multiple technologies including PCR to clone contigs, web-search based approaches, radiation hybrid mapping, and FISH methodology. Markers were screened against 37 YACs and 12 BAC, PAC, or P1 clones to create a physical map of the region. All markers have been blasted to the NCBI database and 30/60 hit 17 additional sequenced clones. 22 markers have been studied on the G3 and 19 on the TNG4 radiation hybrid panels to order the markers. In addition, we have performed FISH on 27 clones to metaphase chromosomes to confirm their map locations. We are in the process of identifying and studying candidate genes in the region and comparing these genes to the syntenic region in murine EAE7 on mouse chromosome 11. This work will aid in the discovery of a susceptibility locus for multiple sclerosis and may be of interest for other diseases such as breast cancer that show frequent amplification events in this chromosomal area.

Program Nr: 2522 from the 1999 ASHG Annual Meeting

Screening endothelin-1 by SSCP analysis for mutations associated with nonsyndromic cleft lip and palate in individuals of Filipino origin. *R.E. Schultz, A. McColley, J.C. Murray.* Dept. of Pediatrics, Univ.of Iowa, Iowa City, IA.

Cleft lip and palate (CL/P) is a congenital abnormality present in approximately 1/1000 live births. About 30% of cases of CL/P are syndromic, with causes such as Mendelian disorders, chromosomal anomalies, or known teratogen exposure. The remaining 70% are nonsyndromic (NS), meaning that the affected individual has no other physical abnormalities or known teratogen exposure. Previous studies have shown linkage for NS CL/P to a locus at 6p23, which contains the candidate gene endothelin-1. Mice knockouts for endothelin-1 show CL/P, and endothelin-1 is also hypothesized to be involved in the epithelial-mesenchymal transformation. Endothelin-1 consists of 5 exons. Primers were designed to screen all of the the coding sequence and the intron-exon boundaries of endothelin-1 for mutations via SSCP analysis. 73 Filipino individuals with nonsyndromic CL/P and both unaffected parents were the samples used for SSCP analysis. A primer to exon 2 identified an SSCP variant. Out of the 73 triads (unaffected parents and affected child), there were 62 in which all three members were homozygous for the wildtype allele. 11 triads contained a G to A mutation at bp 426 of the coding sequence. Of these 11 triads, 8 showed the mutation in both a parent and the affected child, while 3 showed the mutation only in a parent. The p-value for the transmission disequilibrium test was 0.1243, indicating a trend towards significance. The mutation at position 426 was a silent mutation. Because the bp 426 change is a silent mutation, it is less likely that this polymorphism is a disease-causing mutation. However, it is possible that it is linked to a disease-causing mutation elsewhere in the gene, for example in the promoter or an intron, or in a nearby gene. These possibilities should be investigated. Further study on this SSCP variant will be carried out by screening further affected Filipino individuals and their unaffected parents, screening other populations, and sequencing other individuals with the SSCP variant. With a larger population, the results might reach greater significance.

Program Nr: 2523 from the 1999 ASHG Annual Meeting

The autosomal recessive non-syndromic deafness loci, DFNB8 and DFNB10 on human chromosome 21q22.3, are distinct loci. *H.S. Scott[1], A. Berry[2], M. Korostishevsky[2], I. Talior[2], C. Barras[1], C. Gehrig[1], F. Younus[3], A. Veske[4], A. Mohyuddin[3], S. Qasim Mehdi[3], A. Gal[4], J. Kudoh[5], N. Shimizu[5], B. Bonne-Tamir[2], S.E. Antonarakis[1].* 1) Division of Medical Genetics, Univ Geneva Medical School, Geneva, Switzerland; 2) Department of Human Genetics and Molecular Medicine, Sackler School of Medicine, Tel Aviv University, Israel; 3) Biomedical and Genetic Engineering Division, Khan Research Laboratories, Islamabad, Pakistan; 4) Institut fur Humangenetik, Universitats-Krankenhaus Eppendorf, Germany; 5) Department of Molecular Biology, Keio University School of Medicine, Japan.

Deafness affects approximately 1/1,000 newborns. Approximately 50% of early-onset deafness cases are genetic and approximately 50% of these are recessively inherited. Nineteen recessive non-syndromic deafness loci have been identified to date, but only 5 have been cloned. Cloning of deafness genes will shed new light on the biology of the ear, hearing and deafness. Two families with autosomal recessive non-syndromic deafness have previously been independently reported with linkage to the distal chromosome 21q. A Pakistani family (DFNB8) revealed a likely localization telomeric to D21S212 while the disease locus (DFNB10) in a large Palestinian family maps to a 12-cM region in 21q22.3 with homozygosity of only the most telomeric marker, D21S1259. It was not clear if these two loci were in fact the same. By using additional markers (precisely mapped on BAC/cosmid contigs) on critical meioses and additional family members, we have delineated DFNB8 from DFNB10 as being telomeric and centromeric to D21S1885 respectively. This result is perhaps not surprising as the Pakistani and Palestinian kindreds, while both having recessive non-syndromic deafness, are phenotypically different, having childhood-onset and congenital deafness respectively. Several candidate genes for both loci have been excluded by mutation analyses. Ongoing studies will help identify the minimal critical regions for positional cloning efforts.

Program Nr: 2524 from the 1999 ASHG Annual Meeting

Examination of association between *LRP1* and late-onset Alzheimer disease using single nucleotide polymorphisms (SNPs). *W.K. Scott[1], E.H. Lai[2], J.M. Grubber[1], E.R. Martin[1], D.M. Hill[1], S.H. Poulton[1], M.M. Menold[1], L.A. Farrer[3], P.M. Conneally[4], G.W. Small[3], A.M. Saunders[1], A.D. Roses[2], J.R. Gilbert[1], J.L. Haines[5], M.A. Pericak-Vance[1]*. 1) Duke Univ. Medical Center, Durham, NC; 2) GlaxoWellcome Research & Development, RTP, NC; 3) Boston Univ. School of Medicine (LAF); Univ. of California at Los Angeles (GWS); 4) Indiana Univ. School of Medicine, Indianapolis; 5) Vanderbilt Univ. Medical Center, Nashville, TN.

The associations of a 5' tetranucleotide repeat and a non-coding SNP in exon 3 of the low density lipoprotein receptor-related protein (*LRP1*) gene with late-onset AD have been controversial. One potential explanation for these conflicting results is that varying degrees of linkage disequilibrium (LD) exist between these polymorphisms and the pathogenic variation. *LRP1* is an attractive candidate gene due to its function as a receptor for apoE as well as its location in the region on chromosome 12 recently linked to late-onset familial AD. Thus, these conflicting results necessitate more detailed study. The potential variation in LD across this large gene (89 exons) requires that several intragenic markers be examined. We conducted a more thorough examination of association and linkage between *LRP1* and AD by generating SNPs in 2 introns (38 and 78) and 3 exons (44, 54, and 56) for analysis with the previously described exon 3 SNP. To avoid bias by population stratification, we used the S-TDT and SDT family based association methods to test for linkage and association in 183 discordant sibships from late-onset AD families ascertained by Duke, the NIMH AD Genetics Initiative, and the Indiana Univ. AD Cell Repository. Logistic regression was used to examine association in 265 cases of sporadic AD and 276 controls ascertained at Duke and Boston Univ. Family-based tests of association (S-TDT and SDT) failed to detect statistically significant evidence of linkage disequilibrium at any of the 6 SNPs. Logistic regression analysis of the unmatched case-control data set, controlling for age, sex, and APOE, also found no statistically significant evidence for association. These data suggest that *LRP1* is not likely a significant risk factor for AD.

Program Nr: 2525 from the 1999 ASHG Annual Meeting

Fine mapping of the 11q13 Bardet-Biedl Syndrome1 (BBS1) locus and use of the rat EST map to identify BBS1 candidate genes. *M.D. Shastri[1], T.E. Scheetz[1], D. Nishimura[1], A.S. Cornier[2], G.F. Cox[3], A.B. Fulton[4], E.M. Stone[1], V.C. Sheffield[1]*. 1) Pediatrics, HHMI, University of Iowa, Iowa city, IA; 2) Ponce School of Medicine, Puerto Rico, PR; 3) Div. of Genetics, Children's Hospital, Boston, MA; 4) Dept. of Opthalmology, Harvard Medical School, Boston, MA.

Bardet-Biedl Syndrome (BBS) is an autosomal recessive disease characterized by retinal degeneration, polydactyly, obesity, mental retardation, and hypogonadism. BBS is genetically heterogenous showing linkage to 5 loci on chromosomes 2, 3, 11, 15, and 16. BBS1 was initially mapped using small pedigrees to a large interval around PYGM on 11q13 (Leppert et.al., 1994). We have analyzed a number of large families and refined the genetic interval. YACs, BACs, and PACs have been used to construct a physical contig across the BBS1 interval. In addition to the work on BBS1, we are in the process of constructing a dense EST map of the rat genome and are taking advantage of this map for the positional cloning of the BBS1 gene. Comparison of human ESTs in the BBS1 interval from the GeneMap99 with the rat EST placement map from the University of Iowa Rat Gene Discovery and Mapping Project (ratest.uiowa.edu) identifies an area of synteny between rat and human. More than 50 non-redundant rat ESTs were identified in the BBS1 syntenic region. Many of these ESTs were determined to be novel based on BLAST analysis using a threshold E-value of e^{-10}. This work shows that utilization of the rat EST data is an effective approach for identifying novel candidate genes. We are in the process of performing similar analysis on the other known BBS intervals. Comparison of genes between intervals is expected to aid in the identification and prioritization of BBS candidates for mutation screening by identifying genes of similar function. Refernce Leppert, M., et. al., (1994) Nature Genetics 7:108-112.

Program Nr: 2526 from the 1999 ASHG Annual Meeting

Selective Sib Pairs for Mapping QTL in Humans Using Identity in State Versus Identity by Descent. *S.S. Shete.* Epidemiology and Biostatistics, CWRU, Cleveland, OH.

A modification of the Risch-Zhang method of linkage analysis is proposed for two different models. First we consider a model in which the marker locus itself is the trait locus, called the candidate locus model. In the second model we assume that the trait locus and the marker locus are two distinct loci with a recombination fraction of q. These proposed procedures are based on identity in state relations between sib pairs. It is shown that with the proposed modification also, like the Risch-Zhang method, sib pairs with extreme discordant/concordant phenotypic values are more useful and powerful for linkage than sib pairs with intermediate phenotypic values. One of the advantages of the proposed procedures is that we do not need information about the parents and hence it is a good alternative for diseases with late age of onset, where parental information may not be available. A comparison through the number of sib pairs required to have given power is made for both these models with the Risch-Zhang method which is based on the ibd status of the sib pairs. We found that for this allele sharing procedure the iis based method has more power in the case of the candidate locus model, but has less power when the trait locus and the marker locus are two different loci. Possible implications of these results are discussed.

Program Nr: 2527 from the 1999 ASHG Annual Meeting

Linkage analysis of autosomal dominant polycystic kidney disease in the Japanese population. *Y. Shimizu[1], M. Mizoguchi[1,2], T. Kimura[1], A. Yamaki[1], T. Tamura[1], E. Higashihara[3]*. 1) Medical Genetics, Kyorin Univ, Tokyo, Japan; 2) Dept. Nursing, Sch. Health Sci., Tokai Univ; 3) Dept. Urology, Sch. Med., Kyorin Univ.

At least three different genes are thought to be causative for autosomal dominant polycystic kidney disease (ADPKD). They are PKD1 at 16p13.3, PKD2 at 4q21-q23 and PKD3 at the unknown locus. The mutations of PKD1and PKD2 have been reported at a variety of sites but only a small fraction of ADPKD patients. We have performed linkage analysis to clarify the ADPKD genetic heterogeneity in the Japanese population. To assess linkage to PKD1 and PKD2, we used two intragenic (KG8 and I42) and four flanking markers (D16S521, 16AC2.5, CW2, SM7) of PKD1 and six flanking markers (D4S231, D4S1534, D4S1542, D4S1563, D4S41544, D4S414) of PKD2. The analysis showed that 22 families linked to PKD1, one family to PKD2, and two families (family 68 and family 79) linked to neither PKD1 nor PKD2. We found an abnormal band in three out of four patients of family 68 by PCR-SSCP analysis. The direct sequence analysis revealed a nonsense mutation, C12581T (Gln4124stop), in exon 45 in this family except one patient. Thus we assume that the nonsense mutation and an additional unknown sporadic mutation may exist in family 68. In family 79, we obtained the maximum lod scores 2.01 with PKD1 markers and 3.39 with PKD2 markers from linkage analysis and no mutation was found in exons 36 through 46. These findings suggests the existence of the third genetic locus for ADPKD in family 79.

Program Nr: 2528 from the 1999 ASHG Annual Meeting

BIPOLAR AFFECTIVE DISORDER SUSCEPTIBILITY LOCI ON CHROMOSOME 5 AND 12 IN A HOMOGENOUS POPULATION IN QUEBEC. *E. Shink[1], J. Morissette[1], A. Villeneuve[2], L. Bordeleau[1], D. Rochette[3], V. Raymond[1], B. Gagné[1], C. Laprise[1], G. Bouchard[4], M. Plante[1], N. Barden[1].* 1) Neuroscience, CHUL research center, Ste-Foy, Québec, Canada; 2) Clinique Roy-Rousseau, Québec; 3) Complexe Hospitalier de la Sagamie, Chicoutimi, Québec; 4) Université du Québec a Chicoutimi.

A complete genome-wide scan at approximately 11 cM was conducted in a very large multiplex family with bipolar affective disorder from the Saguenay-Lac-St-Jean region of Qubec. We have already reported that this pedigree, which included more than 130 living members, suggests linkage with bipolar affective disorder in one region of chromosome 12 (Z=2.87). Using a more restrictive definition of affected status, the same genome survey pointed to two different regions on chromosome 5. Under this definition, bipolar disorder, type I, schizo-affective disorder, bipolar type and bipolar disorder, type II were considered as affected, whereas single or recurrent major depressive episodes were classified as unknown. All other diagnoses were considered as unaffected. The GENEHUNTER multipoint nonparametric analysis suggested the presence of a susceptibility locus at the 5q33-34 region (NPL score=2.6 with p=0.022). This region, delimited by markers D5S673 and D5S422, was also supported by parametric analysis (Z=2.15) and by SimAPM (p=0.014) at marker D5S412. Additionally, another chromosome 5 area, delimited by D5S406 and D5S432, was pointed too by the affected sib-pair test with p-values less than 5% for these two successive markers. To analyze the linkage heterogeneity, the whole pedigree was broken down into 5 branches. Two of these branches, which were the most linked to the 12q23-24 region, gave also some support for the chromosome 5p loci, whereas the two others informative branches supported the 5q region. Others independent research teams also reported positive finding in these regions: Kelsoe et al. observed positive LOD scores on 5p15 near the dopamine transporter locus and Macciardi et al. found linkage disequilibrium between the GABRA1 gene (5q34-q35) and bipolar disorder. These results suggest that more than one susceptibility locus may be involved in transmission of bipolar disorder in this community.

Program Nr: 2529 from the 1999 ASHG Annual Meeting

Detection of linkage for quantitative trait loci. *D.O. Siegmund, H.-K. Tang.* Dept Statistics, Stanford Univ, Stanford, CA.

We present a self-contained discussion and comparison of strategies for mapping quantitative trait loci, with emphasis on situations where there are thought to be gene/gene or gene/environment interactions. Beginning with a simple general treatment of certain issues of study design that have recently received attention, e.g., genotyping only selected pedigrees, the comparative value of large pedigrees versus sib pairs, we then introduce and study simultaneous and conditional search to detect linkage when there is thought to be gene/gene or gene/environment interaction. By using a particular parameterization of the genetic effects and the framework of local alternatives employed in large sample statistical theory, we obtain simple explicit expressions for asymptotic noncentralilty parameters. From these expressions, with negligible computational effort we can compare the power of different strategies. ~.

Program Nr: 2530 from the 1999 ASHG Annual Meeting

Identification and analysis of polymorphic loci in and around the a**-synuclein and synphilin genes in Parkinson's disease and dementia with Lewy bodies.** *A.B Singleton[1], S. Lincoln[1], D. Dickson[2], J. Hardy[1], M. Farrer[1].* 1) Neurogenetics, Mayo Clinic Jacksonville, Jacksonville, FL; 2) Mayo Clinic Jacksonville, Jacksonville, FL.

The pathobiological significance of a-synuclein has recently become an area of much interest to researchers working in the field of neurodegenerative disease. This protein has long been known to be a component of Alzheimers disease senile plaques and is now known to be a major depositing protein within Lewy bodies. Lewy bodies are seen in numerous neurodegenerative disorders most notably dementia with Lewy bodies (DLB) and Parkinson's disease (PD). Although the physiological function of this protein is unknown the pathogenic importance of the a-synuclein gene has been emphasized by the discovery that it harbors mutations in some rare cases of familial PD. A novel a-synuclein interacting protein, synphilin, has recently been identified and cloned.

In order to find novel single nucleotide polymorphisms in the a-synuclein and synphilin genes we used a direct sequencing approach in twenty mixed PD, DLB and control cases. We have identified two novel polymorphisms in the a-synuclein gene, within and 5' to exon 5. Examination of the synphilin gene demonstrated the presence of 3 informative single nucleotide polymorphisms in exon 1, 25bp 5' to exon 4 and 21bp 5' to exon 6. In addition we have identified a polymorphic $(CA)_{14\text{-}19}$ repeat 5' to exon 5 of this gene.

In order to examine any putative role that the a-synuclein and synphilin genes may have to play in Lewy body disorders we have determined the frequencies of these polymorphisms and a known imperfect repeat in the 5'UTR of the a-synuclein gene in a large series of PD, DLB and control cases.

Program Nr: 2531 from the 1999 ASHG Annual Meeting

SIMPLE: A linkage program that incorporates interference. *Z. Skrivanek[1], M. Irwin[1], S. Lin[1], F.A. Wright[2]*. 1) Department of Statistics, Ohio State University, Columbus, OH; 2) Human Cancer Genetics Program, Ohio State University, Columbus, OH.

Although chiasma interference is known to occur in human meiosis, it is typically ignored in order to facilitate the calculation of multipoint likelihoods in linkage analysis. This gain in computational ease often occurs at the expense of power to accurately construct a genetic map. Linkage analysis programs that have incorporated interference models to date are limited to either small pedigrees or a small number of loci. We present a software package, SIMPLE (Sequential Imputation for Multi Point Linkage Estimation), that can handle both large simple pedigrees and a moderate number of markers and easily incorporates almost any interference model. SIMPLE uses Sequential Imputation, a Monte Carlo method, giving it the power and flexibility to achieve these ends. To demonstrate the advantages of SIMPLE, we perform linkage analysis on simulated data sets from large pedigrees with a moderate number of loci. We model the chiasma interference using the chi-squared model: chiasmata are treated as events from a stationary renewal process with inter-event distances following a chi-squared distribution. We simultaneously analyze the same data with no interference and compare the two methods in terms of power, size and lod scores. The advantages of SIMPLE are the flexibility it offers in facilitating a wide range of interference models and the ability to handle large simple pedigrees and a moderate number of loci at the same time.

Program Nr: 2532 from the 1999 ASHG Annual Meeting

Multipoint linkage analysis of complex traits with Markov chain Monte Carlo linkage analysis. *G. Snow[1], E. Wijsman[1], E. Thompson[1], S. Heath[2].* 1) Biostatistics, University of Washington, Seattle, WA; 2) Rockefeller University, New York, NY.

Lod scores for multipoint linkage analysis can be difficult or impossible to compute for large and/or complex pedigrees when there are many multiallelic markers. Markov chain Monte Carlo (MCMC) methods offer one computationally tractable solution. MCMC methods are based on realization of dependent samples of genotypes, from which desired parameters and likelihood ratios can be estimated. Earlier MCMC samplers for lod score computations are based on single-genotype updating (e.g., the Gibbs sampler), which tends to be relatively inefficient thus limiting practical utility.

Here we show that use of a sampler which simultaneously updates all genotypes at each locus in turn is much more efficient. We also incorporate a mixed-model for the trait locus, thus allowing for a more complex model. We applied this approach to a 101-member simple-structure pedigree with simulated trait and marker data, and compare results to those obtained by Vitesse by combining environmental and polygenic variance. For 4 markers spaced at 20 cM intervals, a linked quantitative trait with major gene heritability .69 and polygenic heritability .15, the peak lod score (5.6) for the MCMC analysis is virtually identical to that obtained by Vitesse (5.7), and both peaks occur at the correct location. However, the MCMC approach provides more accurate localization (1 lod support interval ~50% that provided by analysis with Vitesse) and requires less CPU time. When 10% of the pedigree is unobserved, for 4 markers Vitesse requires 115 minutes vs.20 minutes for the MCMC approach; for 5 markers, Vitesse requires >12 hours, while < 30 minutes are required for the MCMC approach. This demonstrates the practical utility of this approach for multipoint linkage analysis of large pedigrees.

Supported by NIH GM 46255

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Program Nr: 2533 from the 1999 ASHG Annual Meeting

A comparison of the power of haplotype sharing analysis and two locus association analysis for fine-mapping of genes contributing to complex genetic disease. *G.T. Spijker, I.M. Nolte, G.J. te Meerman.* Medical Genetics, University of Groningen, the Netherlands.

Fine-mapping of genes that contribute to complex disease presents a major challenge to geneticists. At a density of genes of perhaps one gene per 15 kb of DNA, association between genetic markers and disease alleles may be insufficient to achieve sufficient accuracy of fine mapping. We propose that systematic haplotype comparison offers an improved perspective on genetic fine mapping, by using all information of haplotype by haplotype comparison. The physical model underlying haplotype similarity is haplotype decay starting from the position of an original mutant allele. It is likely that the highest similarity between patient haplotypes is found at the marker interval containing the risk allele. This similarity is reflected by an excess sharing between pairs of haplotypes containing the risk allele. We have developed an analysis method that systematically investigates this haplotype sharing. We expect this method to be less sensitive to differences in marker informativity than one and two locus association methods, especially when allelic heterogeneity is present. The goal of this study is to investigate the properties of haplotype sharing analysis with regard to power and accuracy of fine mapping and to compare them with one and two locus chi-square association tests. We present results pertaining to empirical and simulated data sets. The independent variables are degree of allelic heterogeneity, marker densities and the number of unknown alleles. Dependent variables are sample size and accuracy of mapping.

Program Nr: 2534 from the 1999 ASHG Annual Meeting

CCR5 Does Not Influence Genetic Susceptibility to Multiple Sclerosis in the Canadian Population. *J.L. Steckley[1], K. Cousin[2], A.D. Sadovnick[3], N. Risch[4], G.C. Ebers[1], The Canadian Collaborative Study Group[5]*. 1) University of Oxford, Department of Clinical Neurology, Radcliffe Infirmary, Woodstock Road, Oxford, England; 2) The Department of Clinical Neurological Sciences, London Health Sciences Centre, University Campus, London, ON,; 3) The Department of Medical Genetics, University of British Columbia, Vancouver, BC; 4) The Department of Genetics, Stanford University, Palo Alto, California.

Chemokines are thought to be important for the establishment of inflammatory processes in putative autoimmune diseases such as multiple sclerosis (MS). There is a growing body of evidence for the involvement of macrophage inflammatory protein (MIP)-1a and its main receptor, CCR5 in the development of MS. A common 32bp deletion (D32bp) in the CCR5 gene prevents CCR5 receptor expression on the surface of cells. There have been a number of attempts to implicate a retroviral etiology in MS and thus D32bp could protect against viral infection. Two-hundred and ninety families with MS that were obtained as part of the Canadian Collaborative Project on Genetic Susceptibility to Multiple Sclerosis (CCPGSMS) were analyzed for the D32bp mutation. We analyzed the D32bp genotype, as well as the genotype of two neighbouring microsatellite markers (D3S4580 and D3S4579) for linkage and linkage disequilibrium with the ASPEX linkage package. Eleven MS patients were found to be homozygous for the D32bp mutation, showing that the absence of the CCR5 receptor on the cell surface does not protect from the development of MS. Further, the transmission disequilibrium test (TDT) showed no preferential transmission of the D32 mutation to affected offspring and linkage analysis excluded the D32bp mutation from contributing to MS susceptibility at a l value of 1.50. There was no excess transmission of alleles to affected offspring for the microsatellite markers and multipoint linkage analysis excluded the CCR5 gene. We conclude that CCR5 polymorphism does not influence susceptibility to MS in the Canadian population.

Program Nr: 2535 from the 1999 ASHG Annual Meeting

Multiple origins of the spinocerebellar ataxia (SCA7) mutation revealed by linkage disequilibrium studies with closely flanking markers, including an intragenic polymorphism ($G^{3145}TG/A^{3145}TG$). *G. Stevanin[1], G. David[1], A. Durr[1], P. Giunti[2], A. Benomar[3], M. Abada-Bendib[4], M.S. Lee[5], A.S. Lebre[1], Y. Agid[1], A. Brice[1]*. 1) INSERM U289, Hopital la Salpetriere, Paris, France; 2) Institute of Neurology, Queen Square, London, UK; 3) Service de Neurologie, Hopital des Specialites, Rabat, Morocco; 4) Service de Neurologie, Hopital de Ben-Aknoum, CHU Alger-Ouest, Algiers, Algeria; 5) Department of Neurology, Yongdong Severance Hospital, Seoul, Korea.

Spinocerebellar ataxia (SCA7) is a neurodegenerative disease characterised by cerebellar ataxia and progressive macular degeneration. The cause of the disease is a (CAG)n repeat expansion in the coding sequence of the SCA7 gene on chromosome 3p. De novo mutations occur on intermediate sized alleles carrying from 28 to 35 CAG repeats. Neomutations explain the persistence of the disease in spite of the great instability of the repeat sequence. This is the biological base for the marked anticipation that characterises this disease that results in the appearance of juvenile onset patients and its extinction within families. This rare disorder has been reported in a large number of countries and ethnic groups. We have selected 41 SCA7 families of different origins and determined the haplotypes segregating with the mutation at several microsatellite markers close to the SCA7 gene as well as at a newly described intragenic polymorphism ($G^{3145}TG/A^{3145}TG$). Four different haplotypes were observed for centromeric markers ($G^{3145}TG/A^{3145}TG$ - D3S1287 - D3S3635) in the majority of the kindreds from 4 different geographical regions: A-2-4 in Korea; A-3-6 in North-Africa, B-3-6 in continental Europe and A-4-6 in UK and USA. The haplotypes segregating in the Jamaican, Filipino, Brazilian and German families were different, and suggest that independent regional founders are at the origin of the SCA7 mutation in each population. Two different haplotypes were observed, however, in two families from the same rural area in central Italy in which de novo SCA7 mutation have been observed. These results suggest the existence of different pools of at-risk chromosomes in this population.

Program Nr: 2536 from the 1999 ASHG Annual Meeting

Stocco dos Santos XLMR Syndrome: Clinical Update and Linkage Analysis. *R.C. Stocco dos Santos[1], L.M. Holmes[2], C.J. Lindsey[3], H.A. Lubs[4], D.T. Horne[2], R.C. Recouso[1], R.E. Stevenson[2], C.E. Schwartz[2].* 1) Laboratorio de Genetica, Instituto Butantan, São Paulo, São Paulo, Brazil; 2) J.C. Self Research Institute, Greenwood Genetic Center, Greenwood, SC,29646; 3) Departamento de Biofisica, UNIFESP, São Paulo, São Paulo, Brazil; 4) Department of Pediatrics, University of Miami School of Medicine, Miami, FL.

The Stocco dos Santos XLMR syndrome was originally described in 1991 (Am J Med Genet 39:133) as a condition in which the affected males had mental retardation, bilateral hip luxation and short stature. Three of the four affected males also had a variant of G6PD. Recently, we re-examined the family. In addition to the original clinical features, the affected males were found to now have kyphosis, seizures, strabismus, hyperactivity and possible premature aging. Linkage analysis was conducted using 4 normal males and 4 carrier females in addition to the 4 affected males. Tight linkage was found between the loci DXS983 and AR (zero recombination with a lod score of 3.13) and the XLMR. Recombination was detected at DXS1003 (Xp11.3) and DXS8077 (Xq21.1) thereby localizing the syndrome to the pericentric region. Furthermore, the female carriers were found to have skewed X-inactivation. Based on the combination of these findings, the XNP gene has been screened for mutations; to date no alterations have been observed. Other potential XLMR genes located in the linkage interval, such as OPHN1, are also being screened for mutations. (Supported in part by NIH grant HD26202 to H.A.L. and C.E.S. and by the South Carolina Department of Disabilities and Special Needs.).

Program Nr: 2537 from the 1999 ASHG Annual Meeting

Genetic Heterogeneity in Carney Complex (OMIM 160980): Contributions of loci at chromosomes 2 and 17 in its genetics. *C.A. Stratakis[1], L.S. Kirschner[1], S.E. Taymans[1], C.J. Vaughan[2], C.J. Hatcher[2], M. Casey[2], J.A. Carney[3], C.T. Basson[2]*. 1) Unit on Genetics & Endocrinology, NICHD, NIH, Bethesda, MD; 2) Div. of Cardiology, Dept. of Medicine, Cornell University Medical College, The New York Hospital, New York, NY; 3) Mayo Clinic, Rochester, MN.

Carney complex (CNC) is an autosomal dominant disorder characterized by spotty skin pigmentation, myxomas of the skin, heart, breast and other sites, and other tumors. Despite its unique phenotype, which suggested a single causative gene, CNC has been linked to two different locations of the human genome. At first, 10 families collected by the National Institutes of Health (NIH) and the Mayo Clinic (MC) were mapped to chromosomal region 2p15-16; subsequently, 5 families [1 from the NIH study and 4 collected from Cornell University (CU)], were mapped to 17q22-24. We reexamined the evidence for linkage to 2p15-16 in 16 families, including 3 previously unreported families, as well as the previously described families from the NIH, MC and CU collection. 150 individuals (84 affected) were genotyped for 11 polymorphic markers on 2p15-16 and for 4 markers on 17q22-24. LOD score analysis demonstrated that 5 families were linked to 17q22-24. In 5 families, haplotype analysis showed at least one recombination with the chromosome 17 locus; 4 of these families showed no recombination with 2 tightly linked markers on 2p15-16 with summed pairwise LOD scores (Z) 3.82 and 3.67 for D2S2156 and CA2, respectively. HOMOG revealed no heterogeneity amongst these 4 families. The maximum summed multipoint LOD score (MMLS) for marker D2S2156 was 3.8; overall, MMLS for 2p15-16 was 4.1 (centered between markers CA-7 and D2S1352). One family had recombinations with both loci; 4 families had no recombinations for either locus or were uninformative. Two families that demonstrated recombinations with 2p15-16 segregated with markers from 17q22-24. These observations confirm locus heterogeneity in CNC. Of 16 families studied, we predict that 7 map to 17q22-24 and 4 to 2p15-16. Four families could not be definitely mapped, and a third locus for CNC in the genome may exist, because one family exhibited recombinations with both loci.

Program Nr: 2538 from the 1999 ASHG Annual Meeting

Lod scores for disease mapping in the presence of marker map uncertainty. *H.M. Stringham, M. Boehnke.* Department of Biostatistics, University of Michigan, Ann Arbor.

Multipoint lod scores are typically calculated for a grid of locus positions, moving the putative disease locus across a fixed genetic marker map. The resulting multipoint lod score curve is then plotted against this map. Changing the order of a set of markers and/or the distances between the markers can make a substantial difference in the lod score curve. Given densely-spaced markers, there may not be a single clearly best marker order, and the maximum lod score and estimated disease gene location may differ depending on which marker order is used. There is need for a method to deal with this uncertainty in the map, provide a single multipoint lod score which accurately reflects the evidence for linkage, and give an appropriate estimate of disease gene location.

We propose three weighted multipoint lod score statistics that make use of information from all plausible marker orders. In each of these statistics, the information conditional on a particular marker order is weighted by the posterior probability of that order. The first statistic is a lod score formed from weighted likelihoods. The second is a weighted average of the lod scores themselves. For the third statistic, we calculate a weighted significance level and then back transform to its lod score equivalent. We illustrate the use of these three lod score statistics with an example and evaluate their properties based on results from simulated data. We consider the type I error, power, and bias in the estimate of location for the disease locus and compare these results to those obtained using the best maximum likelihood map and the true genetic map.

Program Nr: 2539 from the 1999 ASHG Annual Meeting

Efficiency of Various Methodologies for Estimating Penetrance. *M.D. Swartz[1], C.I. Amos[2].* 1) Department of Statistics, Rice University, Houston, TX; 2) Department of Epidemiology, The University of Texas M.D. Anderson Cancer Center, Houston, TX.

Statistical methods to estimate the penetrance of a complex disease include segregation analysis without a linked marker, joint segregation and linkage analysis, and maximizing the LOD score over the penetrance (the MOD approach). For back cross matings, analytic studies [Liang et al., (1996)Genetic Epidemiology 13:575-593, Amos & Rubin (1995) Exp Clin Immunogenet 12:141-155] have compared the efficiency of parameter estimation between combined segregation and linkage and segregation analaysis. These studies showed that the increase in efficiency to estimate the penetrance comparing combined segregation and linkage to segregation analysis depended strongly on the ratio of the penetrance in the carriers versus the noncarriers. The MOD score approach has been suggested whenever ascertainment correction cannot be accomplished, but the MOD score method has been criticized as having low efficiency for parameter estimation. Here we analytically study these three methods of penetrance estimation for an autosomal locus using sib pairs with unknown phase. We study these methods while varying not only the penetrance of the carriers but also the penetrance of the noncarriers. Contrary to statements in the literature, the MOD score approach only performs poorly relative to combined segregation and linkage when the relative penetrance of carriers to noncarriers is low. Thus, for highly penetrant (usually rare) alleles, the MOD score approach provides a reliable estimate of penetrance, yet, for alleles having low penetrance, the MOD score approach performs very poorly. These results can be used for deciding which sampling scheme to use in studying a disease, whether population based with well described ascertainment criteria or registry based with a poorly defined ascertainment process.

Program Nr: 2540 from the 1999 ASHG Annual Meeting

Evidence for extensive linkage disequilibrium on the X chromosome. *P. Taillon-Miller[1], I. Bauer-Sardina[1], N.L. Saccone[2], G. Pilia[3], J. Kere[4], J.P. Rice[2], P-Y. Kwok[1]*. 1) Washington University, Dermatology; 2) Washington University, Psychiatry; 3) University of Cagliari, Pediatrics; 4) University of Helsinki, Department of Medical Genetics.

Evidence for extensive linkage disequilibrium on the X chromosome Patricia Taillon-Miller, Irma Bauer-Sardia, Nancy L. Saccone, Giuseppe Pilia, Juha Kere, John P. Rice and Pui-Yan Kwok Washington University, Dermatology, Psychiatry, University of Cagliari, Pediatrics, University of Helsinki, Department of Medical Genetics To date most investigators have looked at linkage disequilibrium (LD) in humans as a means to discover the cause of a specific genetic disease. As we develop a large number of single nucleotide polymorphism (SNP) markers and begin to look at the genetic origin of complex diseases using association studies it will be important to understand the amount and the location of regions of LD across the genome. To approach this problem we have developed a set of closely spaced (less than 100 kb apart) SNPs markers in Xq25-28. The X chromosome is an ideal region in which to begin these studies. It is a well-characterized chromosome and by genotyping only males, we can easily determine haplotypes across the chromosome. We have typed 90 males in 3 populations; the CEPH, Finnish, and Sardinian populations. Linkage disequilibrium was determined by using pairwise comparisons. We have found 2 extended regions of LD ($p<.0.005$) in all populations examined to date. The first is 550 kb in length in Xq25 and the second in Xq28 (340 kb). In addition, we have also found regions that exhibit no LD giving strong support to the theory that LD is not a monotonic function of the distance between markers but is dependent on the region of the chromosome. . These results are in sharp contrast to recent simulations that have concluded that a useful level of LD is unlikely to extend beyond a distance of 3 kb in the general population.

Program Nr: 2541 from the 1999 ASHG Annual Meeting

Fine mapping of the gene for familial cylindromatosis. *M. Takahashi, G. Bignell, E. Rapley, S. Seal, C. Brown, P. Biggs, W. Warren, M. Stratton.* Cancer Genetics, Inst of Cancer Research, Sutton, Surrey, England.

Familial cylindromatosis is a rare autosomal dominant disease characterised by the development of multiple benign tumours of skin adnexal structures. The gene responsible, *Cyld1*, has been mapped to chromosome 16q12.1-q13 and is likely to be a tumor suppressor gene. Seventeen cylindromatosis families have now been analysed using markers within the *Cyld1* region and all are consistent with linkage to the *Cyld1* region. Therefore there is no evidence for genetic heterogeneity. YAC and PAC contigs have been constructed through the region between D16S517 and D16S416 known to harbor the cylindromatosis gene and new polymorphic microsatellite markers isolated. Further recombinant mapping using these markers has reduced the interval to less than 1Mb. There is some evidence for similarity between segregating haplotypes found in independently ascertained families. More detailed characterisation of this potential allelic association is in progress and may result in further reduction of the interval.

Program Nr: 2542 from the 1999 ASHG Annual Meeting

A study of phenotype-genotype correlation in two DPHL families with different missence mutation in the KCNQ4 gene. *Z. Talebizadeh[1], P.M. Kelley[1], J.B. Kenyon[1], G. Haynatzki[2], M. McGlynn[1], S.D. Smith[1].* 1) Genetics, Boys Town Natl Res Hosp, Omaha, NE; 2) Biochemistry, Creighton Univ, Omaha, NE.

We have studied two kindreds ,family 616 and 2177,with dominant progressive nonsyndromic sensorineural hearing loss. Heteroduplex analysis and sequencing analysis have been used for mutation detection. Two missence mutations, L281S and G285S, in the voltage-gated potassium channel KCNQ4 gene have been detected as the etiology of the hearing loss in these families. The two families were compared with respect to hearing level (dB), taking into account three factors: (1) Ear; (2) Age; and (3) Frequency. We avaraged the data on the two ears for each affected individual to increse the precision of the measurements. A Repeated Measures ANOVA and fitting a linear regression model for each family were used for compairing the respective intercepts and slops. The results have indicated the overall level of hearing loss is not significantly different in two families. However, review of the audiograms revealed considerable differences in age of onset and level of hearing loss, in some affected members. This observation suggested involvement of another factor as a secondary cause that produces more severe hearing loss in these subjects. It has been proven that mutations in Connexin 26 could account for 50% of childhood hearing loss, with a carrier rate of 2.8% (Kelley 1998). Abe et al., (1999) found mutation in Connexin 26 among some families with the A1555G mitochondrial DNA mutation. Individuals with both mutations had more severe hearing loss. Thus, it is reasonable to hypothesize that Connexin 26 mutaions or other nuclear or mitochondrial genes play similar roles in causing the clinical variation in some affected individuals with mutaiton in the KCNQ4 gene. To investigate this hypothesis, we have screened the affected individuals in both families for mutaion in Connexin 26, and known mutaions resulting hearing loss in the mitochondrial DNA.

Program Nr: 2543 from the 1999 ASHG Annual Meeting

Mapping of a gene responsible for dermatitis of NOA (Naruto Research Institute Otsuka Atrichia) mice, an animal model of allergic dermatitis. *M. TAMARI[1], O. WATANABE[1], Y. ONOUCHI[1], K. NATORI[1], Y. SHIOMOTO[2], S. KUBO[2], Y. NAKAMURA[1]*. 1) Laboratory of Molecular Medicine, Institute of Medical Science, University of Tokyo,MINATO-ku, Tokyo, Japan; 2) 2Naruto Research Institute, Otsuka Pharmaceutical Factory, Inc., Naruto, Tokushima, Japan.

Atopy and atopic disorders are considered to be multifactorial diseases caused by abnormalities in the immune system. Although a large number of genetic and immunological studies of atopic disorders have been performed, genes predisposing to or developing these diseases are still not well understood because of the etiological complexity in humans. The NOA (Naruto Research Institute Otsuka Atrichia) mouse is an animal model of allergic or atopic dermatitis, a condition characterized by ulcerative skin lesions with accumulation of mast cells and increased serum IgE. These features of the murine disease closely resemble human atopy and atopic disorders. We performed linkage analysis in NOA back-cross progeny, as a step toward identifying and isolating a gene responsible for the NOA phenotype. We crossed NOA mice with five other murine strains (C57BL/6J, IQI, C3H/HeJ, DBA/2J, and BALB/cByJ) and then bred back-cross animals. Using 241 microsatellite markers, we scanned the entire genome of 559 N2 offspring from the five parental strains. Linkage analysis revealed a significant association between ulcerative skin lesions and markers on murine chromosome 14. Statistical analysis indicated the critical region to be the vicinity of D14Mit236 and D14Mit160 ($c2=86.89$, $p<0.001$ for D14Mit236 and $c2=86.62$, $p<0.001$ for D14Mit160). Since this 15-cM region of murine chromosome 14 must contain dozens of genes, it will be useful to construct a more detailed genetic map with a view toward positional cloning of the putative dermatitis-associated gene.

Program Nr: 2544 from the 1999 ASHG Annual Meeting

Identification and genetic mapping of zebrafish circadian clock mutants. *Y. Tan, J. DeBruyne, M.W. Hurd, D.E. Wells, G.M. Cahill.* Biology and Biochemistry, University of Houston, Houston, TX.

We have initiated a screen for dominant mutations that affect the periodicity of behavioral circadian rhythms of larval zebrafish. Random point mutations were induced in male spermatogonial stem cells using N-ethyl-N-nitrosourea. Treated males were then crossed to wild type females, and the resulting (G1) progeny are each heterozygous for a unique set of mutations. The swimming activity in constant conditions of G1 larvae (days 10-17 post fertilization) was measured continuously with an automated video image analysis system, and the average time of the circadian activity peaks from days 5-6 was measured. Among the first 1275 G1 individuals screened, we have identified two mutants that transmit short period phenotypes to their progeny. When these G1 mutants were outcrossed to wild type, the distribution of F1 period phenotypes was consistent with a 1:1 ratio. F2 progeny generated from an F1 mutant intercross had a phenotype distribution ratio consistent with 1:2:1. This is consistent with a semi-dominant mutation. Periods of heterozygous individuals for either mutation were shortened by 0.5-1 hours and the periods of homozygous individuals were shortened by 1-1.5 hours. We tested the effect of one mutation on the period of melatonin production rhythms from cultured pineal glands. Pineals from homozygotes had a significantly shorter period (23.8 0.1 hours, n=7) than pineals from wild type siblings (24.4 0.1 hours, n=7). This indicates that this mutation affects circadian rhythmicity at the tissue level, as well as the behavioral level. Linkage mapping using simple sequence length polymorphisms indicates that one mutation is on linkage group 20 close to the centromere while the other mutation has been narrowed down to a 4cM region on linkage group 7. Together, these findings demonstrate that behavioral rhythmicity provides an efficient screen for zebrafish clock mutants. This work was funded by grants from AFOSR and the Texas Advanced Research Program.

Program Nr: 2545 from the 1999 ASHG Annual Meeting

Computer prediction of optimal temperature and gradient conditions for mutation detection by DHPLC. *P.D. Taylor, D.T. Gjerde, J. Walter, A. Kuklin, R. Haefele.* Transgenomic, Inc., San Jose, CA.

Denaturing high performance liquid chromatography (DHPLC) is a fully automated technique for scanning for novel mutations. The mutant and wild-type sequences are first hybridized to form a heteroduplex containing one or more mismatched bases and then injected onto a polymeric cartridge at a temperature which is slightly denaturing, in order to separate the heteroduplex from the homoduplex. The method is more appropriately termed temperature modulated heteroduplex analysis (TMHA) and takes around 6 minutes per sample. The optimal temperature at which the entire fragment may be scanned for novel mutations, varies with the sequence of the sample, typically within the range of 54 to 68^{o}C. This temperature can be determined experimentally by systematic analysis at 1^{o}C increments although this is time consuming. Alternatively the temperature can be calculated directly from the sequence, using the Fixman-Friere modification of the Poland algorithm. The parameters used in this model have been re-determined for the solvent conditions used for TMHA and applied in a computer program operating in the Windows® environment. The temperatures predicted by this computer program are compared here with those obtained experimentally. The program also predicts more than one temperature when this is required to obtain complete coverage of a sequence which has highly disparate melting domains. Finally, the gradient composition of the two eluents required for separation at the selected temperature is predicted and compared with experimental results.

Program Nr: 2546 from the 1999 ASHG Annual Meeting

Genetic dissection of metabolic pathways. *B.A. Thiel[1], M. Stoll[2], A.W. Cowley[2], A.E. Kwitek-Black[2], H.J. Jacob[2], N.J. Schork[1,3]*. 1) Dept. of Epidemiology/Biostatistics, Case Western Reserve Univ, Cleveland, OH; 2) Department of Physiology, The Medical College of Wisconsin, Milwaukee, WI; 3) The Genset Corporation, La Jolla, CA.

The translation of genotypic variability into high level phenotypic differences is of tremendous interest in contemporary genetics research. Given that a single gene may have an effect on a whole class of measureable phenotypes, analyzing these phenotypes together may provide more compelling insight into gene function than analyzing any one trait individually. In an effort to try and understand the genetic components of multiple phenotypes in complex physiologic pathways, we have used data from genetic crosses using rats to look at the relationships between multiple trait variables and marker genotypes across the genome. These types of analyses are also applicable to, and have been implemented in, human studies.

Our analysis methods involved the use of factor analysis, principal components analysis and cluster analysis to explore the relationships between multiple traits. Our methods also allow one to take advantage of graphical techniques to depict physiological heirarchies under genetic control. We will discuss the assumptions and limitations of these statistical methods for detecting genetic effects.

Program Nr: 2547 from the 1999 ASHG Annual Meeting

A two-stage test of transmission/disequilibrium for quantitative traits in pedigree data. *H.K. Tiwari*[1], *R.C. Elston*[1], *V. George*[2]. 1) Dept Epidemiology/Biostatistic, Case Western Univ, Cleveland, OH; 2) Medical College of Wisconsin, Milwaukee, WI.

The transmission/disequilibrium test (TDT), originally proposed by Spielman et al. for binary traits, is a powerful method for detecting linkage between a marker locus and a trait locus in the presence of allelic association. The TDT uses information on the parent-to-offspring transmission status of the associated allele at a marker locus to assess linkage or association in the presence of the other. All TDT methods are valid tests for linkage, but they only have power in the presence of population association. Recently, George et al. proposed a regression-based TDT for linkage between a marker locus and a quantitative trait locus by modeling the trait as the dependent variable and the transmission status as one of the independent variables, along with other predictors and confounders, in a linear regression model. We propose a two-stage regression-based TDT procedure, specifically for quantitative traits from pedigree data, in which at the first stage a test of population association is performed, and then, if significant association is found, a test for linkage is performed at the second stage. The association test is performed using all members in the pedigree, whereas the linkage test in the second stage is performed using only the informative offspring. We investigate the statistical properties of the procedure by simulating markers, having various levels of association with the trait, at different recombination fractions from the trait locus. The two-stage procedure leads to more definitive conclusions regarding association and linkage, and its power, relative to the single-stage procedure, increases as the number of alleles at the marker locus increases.

Program Nr: 2548 from the 1999 ASHG Annual Meeting

A Gene Responsible for Paroxysmal Kinesigenic Choreoathetosis Mapped to Chromosome 16p11.2-q12.1. *H. Tomita[1], K. Yamada[1], N. Niikawa[1], Y. Nakane[2], S. Nagamitsu[3], T. Matsuishi[3], K. Wakui[4], Y. Fukushima[4], N. Kato[5]*. 1) Dept. of Human Genetics, Nagasaki Univ. School of Medicine, Nagasaki, Japan; 2) Dept. of Neuropsychiatry, Nagasaki Univ. School of Medicine., Nagasaki, Japan; 3) Dept. of Pediatrics and Child Health, Kurume Univ. School of Medicine., Kurume, Japan; 4) Dept. of Hygiene and Medical Genetics, Shinshu Univ. School of Medicine, Matsumoto, Japan; 5) Dept. of Neuropsychiatry, Faculty of Medicine., Univ. of Tokyo, Tokyo, Japan.

Paroxysmal kinesigenic choreoathetosis (PKC) [MIM 128200], a major form of paroxysmal dyskinesias, is characterized by brief attacks of involuntary movements induced by sudden voluntary movements. Although another form, paroxysmal dystonic choreoathetosis, was mapped to 2q33-35, the localization of the PKC gene has not been known. To localize it, we performed linkage analysis on eight Japanese families with autosomal dominant PKC. Two-point linkage analysis provided a maximum LOD score of 10.27 (recombination fraction = 0.00, penetrance; $p = 0.7$) at *D16S3081*, and a maximum multipoint LOD score for a subset of markers was calculated to be 11.51 ($p = 0.8$) at *D16S3080*. Haplotype analysis defined the disease locus within a region of ~12.4 cM between *D16S3093* and *D16S416*. To determine the chromosomal localization, we isolated P1-derived artificial chromosome clones 104-G-3 and 83-D-21 that correspond to the *D16S3093* and *D16S416* loci, and mapped them by FISH to 16p11.2 and 16q12.1, respectively. Thus, the PKC critical region (PKCR) in the eight families studied is assigned to 16p11.2-q12.1. The PKCR overlaps the region responsible for infantile convulsion and paroxysmal choreoathetosis [MIM 602066], a recently recognized clinical entity as a combination of benign infantile convulsion and non-kinesigenic form of paroxysmal dyskinesias.

Program Nr: 2549 from the 1999 ASHG Annual Meeting

One SNP - two alleles - three different complex diseases. *A.H. Torinsson[1], S. Nilsson[3], L. Samuelsson[1], A. Gudjònsdottir[2], B. Hallberg[1], T. Martinsson[1], H. Ascher[2], B. Kristiansson[2], L.M. Sollid[4], J. Ek[5], J. Wahlström[1].* 1) Clinical Genetics, SU/Östra sjukhuset, Göteborg, Sweden; 2) Peadiatrics, SU/Östra sjukhuset, Göteborg, Sweden; 3) Chalmers University of Technology, Göteborg, Sweden; 4) Inst. of Transplantational Immunology, Oslo, Norway; 5) Buskerud Central Hospital, Drammen, Norway.

Insulin Dependent Diabetes Mellitius(IDDM),Coeliac disease(CD) and Multiple Sclerosis(MS) are three complex diseases who all show allelic association to an A/G substitution at the +49 position of the Cytotoxic T-lymphocyte Associated(CTLA4)gene. While several studies show association between IDDM and the G-allele of this polymorphism, association to CD has been described to the A-allele, [Djilali-Saiah 1998]. This association with CD is now confirmed by our group in a Scandinavian population. In our study, Linkage and association analysis by the transmission disequilibrium test(TDT) were used on a total of 108 nuclear families. The TDT showed 115 trans- mitted vs. 78 non-transmitted A-alleles ($p<0.005$). Our results and the other studies all point to one conclusion; true disease gene associations. Interestingly since some of these IDDM and CD associations have been shown in the same population the association to the different alleles means that the potential gene or genes that are involved at 2q33 have different functions in the two diseases. There's an even more intriguing situation when one considers the recent results from a Norwegian study on MS. An association only to the heterozygote(A/G) was shown. Does this mean that perhaps one would need both of these factors interacting to show predisposition to MS?

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Program Nr: 2550 from the 1999 ASHG Annual Meeting

A transmission disequilibrium test for discrete and continuous traits. *D.L. Tritchler[1, 3], S.B. Bull[2, 3], Y. Liu[3]*. 1) Ontario Cancer Institute; 2) Samuel Lunenfeld Research Institute of Mount Sinai Hospital; 3) Department of Public Health Sciences, University of Toronto, Toronto, Ontario, Canada.

A transmission disequilibrium test is derived for traits following any statistical distribution belonging to the exponential family, which includes the normal, Poisson, binomial, gamma, and inverse Gaussian distributions. A general score test statistic is derived which handles all the above cases. The score statistic is linear and leads to an analysis using regression methods, which facilitates outlier detection and model criticism. The model is flexible enough to handle additive and dominance effects, transmission distortion, covariates, and gene-gene and gene-environment interaction. Designs in which observations are sampled based on trait value are accommodated.

Program Nr: 2551 from the 1999 ASHG Annual Meeting

Detection of linkage disequilibrium using information from unlinked regions. *A.M Tsalenko, N.J Cox.* Department of Human Genetics, The University of Chicago, Chicago, IL.

It has been suggested that genome-wide linkage disequilibrium/association (LD/A) studies will become more prominent in the identification of susceptibility loci for common, complex disorders as denser marker maps are developed. The power to detect linkage in the presence of LD is a complex function of the linkage disequilibrium between the susceptibility allele and the marker allele examined, the correspondence between the frequencies of these two alleles, and the magnitude of the contribution the susceptibility locus makes to the liability to disease. In order to determine whether considering the contribution from two or more unlinked regions at a time might improve the power to detect susceptibility loci using LD/A mapping, we have developed a simple screening approach for considering the contributions from two unlinked markers simultaneously. Consider two bi-allelic markers, and the 3x3 matrix corresponding to the genotypes of affected individuals at these two markers. The deviation of the observed matrix from the null matrix computed using the allele frequencies of the markers can be used to determine whether there is evidence for LD and interaction between the markers. We simulated data using two interacting loci and two additional independent susceptibility loci, using recessive models for both the two loci with the epistatic interaction and the two additional independent susceptibility loci (total Kp = 0.13, 0.06 for the epistatic and 0.07 for the independent loci), and compared results of single locus TDT analyses and analyses of the 3x3 matrix for linkage disequilibrium (D') between marker and disease susceptibility alleles ranging from 0.0 to 1.0. We found that the deviation of the 3x3 matrix from its null expectation (under no LD) was detected at lower values of LD than was significant overtransmission of the associated allele via TDT, at least for these models. Moreover, the matrices behave differently in the case of interacting and non-interacting loci. Supported by 1R01 DK55889-01, HL49596 and HL56399.

Program Nr: 2552 from the 1999 ASHG Annual Meeting

Linkage analysis of hemiplegic migraine loci and ChrXq28 in 29 families with familial migraine. *P. van Galen[1], K. Gardner[3], J. Pascual[2], M. Barmada[1], J. Badger[3], M. Soso[3], E. Hoffman[4].* 1) Department of Human Genetics, University of Pittsburgh, PA; 2) Department of Neurology, University Hospital Marques de Valdecilla Santander, Spain; 3) Department of Neurology, University of Pittsburgh, Pittsburgh, PA; 4) Children's National Medical Center,Washington, DC.

Objective: Evaluate familial migraine (with and without aura) for evidence of linkage to the dominant familial hemiplegic migraine (FHM) loci and to a possible X-linked familial migraine locus. Background: Linkage has been previously established for FHM to Chr19p13, Chr1q21-23 and Chr1q31. An X-linked dominant locus spanning a large region around Xq28 has also been suggested for familial migraine. Methods: Probands of 29 migraine families with and without aura were ascertained from Spain (16 families, 69 affected patients) and USA (13 families, 40 affected patients) for a total of 85 sibpairs by neurologists (JP,KG,MS). Additional family members were evaluated directly (Spain) or by questionnaire and clinic or phone interview (USA). DNA was extracted from blood specimens and genotyped with markers D1S422, D1S413, D1S2745, D1S2707, D1S2635, D19S1150, D19S394, DXS1123, DXS1001, DXS1192 and DXS8043 from the four regions using standard PCR, gel electrophoresis and autoradiograms. Alleles were scored, aligned and several different methods of linkage analysis were used, including two-point VITESSE and MMLS with varying penetrance, phenocopy rate and inheritance models; multipoint GENEHUNTER and HOMOG. Results: Of the 29 families evaluated, two point analysis showed a highest LOD of 1.38, theta = 0.0 in a single family for region Chr1q21-23, using a recessive, 50% penetrant model, phenocopy 0%. The highest NPL score for a single family was 1.4 and 1.26, p values 0.043 for markers D1S2635 and D1S2707 at the proximal Chr1q21-23 FHM locus. The highest p value for combined families was 0.0885 at the Chr1q21-23 locus. Conclusion: There is no evidence of significant linkage among 29 familial migraine families to any of the three FHM loci or to the ChrX28 region.

Program Nr: 2553 from the 1999 ASHG Annual Meeting

Study of the apolipoprotein E promoter for Alzheimer's disease in a French population. *P. Verpillat[1], L. Zurutuza[2], G. Raux[2], D. Hannequin[2], A. Brice[3], D. Campion[2], F. Clerget-Darpoux[1], T. Frebourg[2]*. 1) Epidémiologie génétique, INSERM U155, Paris, France; 2) INSERM EPI 9906, Faculté de Médecine et de Pharmacie, IFRMP Rouen, France; 3) INSERM U289, Hôpital de la Salpêtrière, Paris, France.

The apolipoprotein E gene (APOE) is an uncontested genetic risk factor in Alzheimer's disease (AD). The involvement of his promoter has recently been suggested by several studies. Indeed, three polymorphisms of the APOE regulatory region (-186 G/T, -427 C/T and -491 A/T) have been found associated with AD even after adjustment on the APOE status. We analyzed the association of these three promoter polymorphisms on AD in a large French case-control study (388 AD cases and 386 controls). We found that the -427 T and -491 A alleles were associated with an increased risk of developing AD, but not the -186 G/T polymorphism. However, a strong linkage disequilibrium was observed between the alleles of these promoter polymorphisms and the APOE alleles. Consequently, association was retested after adjustment on APOE. Then, only the association with the -427 T allele remained significant. Our study reinforce the idea of a role of the APOE promoter in AD. However, it is important to emphasize that the observed associated alleles differ from one study to another. This may be explained by the different geographical origin of the used samples and by the difference in the linkage disequilibrium found between the alleles of the promoter polymorphisms and the ones of the APOE gene.

Program Nr: 2554 from the 1999 ASHG Annual Meeting

A new linkage analysis method for complex disorders based on multiple sets of data. *V.J. Vieland[1], K. Wang[2], J. Huang[1]*. 1) Univ Iowa, Iowa City, IA; 2) Univ Alabama at Birmingham.

For complex disorders, power to detect linkage in one set of data may be low, and power in follow-up samples may be lower. It is therefore essential to have rigorous methods for 'meta-analysis' based on multiple sets of data. We have developed a novel sequential Bayesian (SB) approach, based on sequential access to data collected at different sites or points in time. Using a prior distribution for the recombination fraction q based on the genetic map, a marginal posterior density for q is estimated while allowing for locus heterogeneity and other covariates. This posterior density is then used as the prior in analyzing a subsequent set of data, in which covariates can be independently modeled. The process can be applied repeatedly as new data become available. This provides a computationally efficient way to model important covariates within data sets while allowing them to vary across data sets. The result can be summarized by the posterior probability of linkage (PPL). Based on simulation of ASP data, we show that the PPL has better power to detect linkage, compared to the standard heterogeneity lod (HLOD), when follow-up data sets have a small proportion of "linked" families. The table shows power based on two data sets (D_1, D_2) of 100 ASP's each, for a simple dominant model (p=.01; penetrance=.5), as a function of the proportion of linked families a_i in D_i. Results are shown for a 0.001 size test; a_1=50%.

Power as a function of the proportion of linked families a2 in D_2

	a2=0.5	a2=0.4	a2=0.3	a2=0.2	a2=0.1
PPL	0.94	0.86	0.74	0.64	0.55
HLOD	0.93	0.84	0.69	0.53	0.35

These results illustrate (i) that for complex disorders, power can actually drop as the sample size increases; and (ii) that the PPL can be a more robust tool for linkage detection in such circumstances.

Program Nr: 2555 from the 1999 ASHG Annual Meeting

Analysis of the human T locus as a candidate gene for neural tube defects. *K.D. Viles[1], C. Drake[1], E. Melvin[1], A. Franklin[1], T.M. George[1], J.M. Vance[1], M.A. Pericak-Vance[1], J. Nye[2], J. Gilbert[1], M.C. Speer[1], NTD Collaborative Group[1]*. 1) Dept Medicine/Med Genetics, Duke Univ Medical Ctr, Durham, NC; 2) Northwestern University Medical School, Dept. of Pediatrics, Chicago , IL.

Neural tube defects (NTDs) are among the most common birth defects. Mouse mutants that show NTDs provide good models for human NTD candidate gene investigation. The T (Brachyury) mutant mouse is one such model. The protein product of the T gene is critical for axis elongation, and heterozygous T mutants have short or absent tails due to abnormal posterior mesoderm development (Morrison, et al 1996). DNA was obtained from ninety-six caucasian patients- seventy-two sporadic cases and twenty-four multiplex cases- affected with lumbosacral myelomeningocele. This DNA was used to examine the human homolog T gene for its possible role as a susceptibility factor for NTDs. Eighty normal, causcasian, unrelated, control samples were also included in the analysis. Primers flanking each of the eight exons were designed from the published human T gene sequence. Each exon was amplified using pcr and analyzed for variants using single-strand conformation polymorphism. Each product showing a mobility shift was sequenced to determine the nucleotide change. All previously reported polymorphisms (Papapetrou et al 1997, Papapetrou et al 1999), including those in exons two, three, seven, and eight, were detected. The DNA sequencing identified a C363T nucleotide change in exon two and a G1176A change in exon eight. Neither of these resulted in an amino acid change. In exon seven, a rare C1013T (Ala338Val) variation was detected in one of the sporadic cases and not at all in the multiplex cases or in the controls. A second variant in exon eight was also detected. This A1106G (Asn369Ser) transition was seen in 15% of the case samples and 21% of the control samples. A third G1066A (Gly356Ser) change in exon eight is still being examined. Further analysis of this gene, including a novel variant in exon five, is in process.

Program Nr: 2556 from the 1999 ASHG Annual Meeting

Linkage of X-linked Myopathy with Excessive Autophagy (XMEA) to Xq28. *L. Villard[1], V. des Portes[2], N. Levy[1], J.P. Louboutin[3], D. Recan[2], M. Coquet[4], B. Chabrol[5], D. Figarella-Branger[5], J. Chelly[2], J.F. Pellissier[5], M. Fontes[1]*. 1) INSERM U491, Fac de Médecine La Timone, Marseille, France; 2) INSERM U129, Fac de Médecine Cochin, 75014 Paris; 3) CJF INSERM 96-01, Hôpital GR Laennec, Nantes; 4) Lab d'Anatomie Pathologique, Hôpital Pellegrin, Bordeaux; 5) Lab de Biopathologie Nerveuse et Musculaire, Fac de Médecine La Timone, Marseille.

X-linked myopathy with excessive autophagy (XMEA, MIM 310440) is a rare inherited mild myopathy segregating as an X-linked trait. This condition is characterized by juvenile onset and slow progression of the disorder, which seems to predominantly affect proximal muscles. Upon histological studies, the muscle does not display acute necrosis but shows an excess of autophagic processes and exocytosis of the phagocytosed material. This condition is named X-linked Myopathy with Excessive Autophagy (XMEA). So far, no extensive genetic analysis of this disorder has been performed, even though a potential lod score to the tip of long arm of X chromosome has been suggested by Saviranta et al. (Zmax=0.9). We have used 32 polymorphic markers spanning the entire X chromosome to exclude most of the chromosome except the Xq28 region in a large XMEA family. Using three additional families for linkage analysis, we have obtained a significant two-point lod score with marker DXS1183 (Z=2.69 at theta=0). A proximal recombinant has been detected between DXS1193 and DXS8011, which places the XMEA locus between DXS1193 and the telomere of the long arm of the X chromosome. Multipoint linkage analysis confirmed the assignment of the disease locus with a maximal lod score of 2.74 obtained at recombination fraction zero. Linkage of XMEA to the Xq28 region is thus firmly established. Several other inherited diseases with muscular involvment have been localized to this region of the human X chromosome and in particular the Emery-Dreifuss muscular dystrophy. We have ruled out the Emery-Dreifuss muscular dystrophy to be allelic with XMEA by direct sequencing of the emerin gene in three of our families.

Program Nr: 2557 from the 1999 ASHG Annual Meeting

Genomic mapping of a fourth gene involved in familial hypercholesterolemia. *L. Villéger[1], M. Varret[1], J-P. Rabès[1], B. Saint-Jore[1], A. Cenarro[2], M. Devillers[1], M. Krempf[3], M.J. Kotze[4], G.M. Kostner[5], M. Farnier[3], M. Martinez[1], C. Junien[1], C. Boileau[1]*. 1) INSERM U383 and U358, Paris, France; 2) Universidad de Zaragoza, Spain; 3) CHU Hôtel Dieu, Nantes and Point Médical, Dijon, France; 4) MRC Cape Heart Group, Tygerberg, South Africa; 5) University of Graz, Austria.

Autosomal Dominant Hypercholesterolemia (ADH) is one of the most common hereditary diseases, characterized by a selective increase of LDL particles, giving rise to premature mortality from cardiovascular complications. ADH results from molecular defects in either of two genes: mutations in the LDLR gene (FH1) lead to FH (Familial Hypercholesterolemia), and 3 mutations in the APOB gene (FH2) lead to FDB (Familial ligand-Defective apolipoprotein B-100). We showed the involvement of a third gene in ADH named FH3 and we localized it at 1p32-p34.1. The genetic linkage homogeneity test at the FH3 locus estimated that 73% out of the 13 nonLDLR/nonAPOB ADH families we studied were not linked to this locus, but to a fourth gene (or group of genes): FH4. We identified a large French ADH family (HC6) in which the involvement of the LDLR, APOB and FH3 genes had been excluded by using both genetic and biochemical approaches, suggesting the existence of this fourth locus. We undertook the identification of the FH4 locus using linkage analysis in family HC6. First, a candidate region approach was used: 30 candidate genes encoding proteins involved in cholesterol metabolism were tested, and linkage was excluded between theses markers and ADH in the family. Then, we undertook an exclusion mapping approach: 220 new microsatellite markers located throughout the genome were tested. To date, 80% of the genome has been excluded and a suggestive linkage was obtained with a Lod score of 2.6 (q=0). Suggestive linkage was also obtained for 3 other nonLDLR/nonAPOB/nonFH3 families [respective Lod scores of 1.17, 0.56 and 0.30 (q=0)] conforting the localization of the FH4 locus in this genomic region. Finally, a critical region of 4.3 cM was defined which probably contains the FH4 gene.

Program Nr: 2558 from the 1999 ASHG Annual Meeting

Autosomal Recessive Distal Spinal Muscular Atrophy with Diaphragmatic Palsy in Childhood is not linked to 5q13-SMA locus. *L. Viollet[1], A. Barois[2], P. Burlet[1], S. Bertrandy[1], E. Vial[1], S. Lefebvre[1], A. Munnich[1]*. 1) INSERM U-393, Hopital Necker, Paris, France; 2) Service de Pediatrie, Hopital Raymond Poincare, 92380 Garches, France.

Distal Spinal Muscular Atrophy with Diaphragmatic Palsy (DSMADP) in childhood is a rare neuromuscular disorder characterized by progressive motor weakness predominating on the distal part of the limbs and involving diaphragmatic dysfunction. Although fatal neonatal cases have been previously described, DSMADP with later onset and survival has not been reported. Symptoms begin after the age of one year, with a disability to walk, progressive course and relative stabilization in adulthood. Symmetric distal motor weakness and amyotrophy predominates in lower limbs and is associated with a later proximal involvement, causing walking missing by the age of five and serious orthopedic complications, such as joint contractures, clubfeet, clawhands and kyphoscoliosis. By contrast to Proximal Spinal Muscular Atrophy (SMA), severe and evolutive diaphragmatic palsy is constant, leading to restrictive respiratory insufficiency, requiring respiratory assistance. Electrophysiological nerve studies show evidence of pure motor neurogenic disorder, of "spinal" type. We report here a pedigree of three DSMADP children born to unaffected parents, consistent with autosomal recessive inheritance. No deletion of SMN exon 7-responsible for Proximal SMA- was detected in the affected cases. Analysis of DNA markers flanking the SMN locus on chromosome 5q13 revealed that two of the affected sibs were discordant for their haplotypes at the SMN locus. These data support the view that, in this family, DSMADP is not linked to chromosome 5q13. Recruitment of more informative DSMADP families will allow to undertake the genetic mapping of the disease and identification of the disease gene will hopefully help understanding the mechanisms of anterior horn cell degeneration in spinal muscular atrophies.

Program Nr: 2559 from the 1999 ASHG Annual Meeting

Genetic Mapping of Multiple Sclerosis in a Large American Pedigree. *E. Vitale[1, 5], M. Devoto[2], R. Sun[1], C. Rohowsky-Kochan[3], D. Nathanson[4], M. Schwalb[1], S. Cook[3].* 1) Dept Microbiology & Molecular Genetics, UMDNJ-New Jersey Medical School, Newark, NJ; 2) University of Genova, Genova, Italy; 3) Dept Neurosciences UMDNJ-New Jersey Medical School, Newark NJ; 4) Dept Neuroscience Sacred Heart Hospital Allentown, PA; 5) Institute of Molecular Genetics , CNR Alghero (SS), Italy.

There is evidence supporting the view that multiple sclerosis (MS) is a complex trait determined by genetic, environmental and autoimmune factors. Association and/or linkage studies to candidate genes have produced many reports of significant genetic effects including those for the major histocompatability complex (MHC), immunoglobulin heavy chain (IgH), T-cell receptor (TCR) and myelin basic protein (MBP) loci. With the exception of MHC, however these results have been difficult to replicate beyond isolated populations. We identified a large pedigree from Pennsylvania, with segregation of MS compatible with a fully penetrant dominant transmission with either an autosomal or X-linked mode of inheritance. We have collected and carefully phenotypically characterized 16 members from this family. Seven members show the typical findings in MS using clinical and magnetic resonance scanning (MRI) criteria. This family presents a unique opportunity to search for genes predisposing to MS by linkage analysis. Analysis of chromosome 6 has already excluded linkage to the HLA region. A first-stage genome screen using markers spaced at an average distance of 10-15 cM produced several lod-scores larger than 1, namely on chromosomes 1, 2, 3, 8, 10, 11, 12, 14, 16, 17, 18, 19. These regions are now being saturated with more markers in order to identify the region of true positive linkage.

Program Nr: 2560 from the 1999 ASHG Annual Meeting

Refined mapping and mutation screening of candidate genes for Welander distal myopathy. *D. von Tell[1,2], A-C. Thelander[1,2], M. Anvret[1], G. Ahlberg[1,2].* 1) Molecular Medicine, Karolinska Hospital, Stockholm, Sweden; 2) Clinical Neuroscience, Karolinska Hospital, Stockholm, Sweden.

Objective: Narrowing of the candidate gene region by refined mapping and screening of candidate genes for Welander distal myopathy(WDM). Background: WDM is a well-described autosomal dominant distal myopathy that is mainly seen in Sweden. Onset is late, between the ages of 40 and 60 and typical manifestations include weakness of long extensor muscles of hands and feet. Cardiac involvement is not seen and patients have normal life expectancy. Linkage studies have mapped the disease locus to chromosome 2p13. The region has been restricted to 2.4 cM through recombinations and a common shared haplotype. Material and Methods: DNA has been isolated from peripheral leukocytes from 122 individuals, 62 affected (9 families). Additional microsatellite markers were genotyped. Candidate genes were screened for mutations by single stranded conformation polymorphisms and direct fluorescent automated sequencing of the PCR products. Results: Recombinations for dysferlin intragenic microsatellite markers reduce the candidate region and exclude dysferlin as a candidate gene. Mutation screening was performed for the following candidate genes: dynactin (DCTN), dysferlin (DYSF), rab1 (RAB1), and adducin (ADD2). Antibodies towards dysferlin were used in Western blotting to screen for defective dysferlin protein product but no abnormalities were found. Discussion: Through new recombinations with markers CY172-H32 and 104-sat the region of the gene locus can further be narrowed down to below 2.4 cM. Further, there are several SNPs within our candidate region that will be investigated in order to minimize the region. No mutations have so far been identified within the screened candidate genes. A number of highly interesting candidate genes and ESTs with neuromuscular expression pattern remain to be screened within the candidate region. A positional cloning effort has been initiated.

Program Nr: 2561 from the 1999 ASHG Annual Meeting

Demonstration of a founder effect in British and Danish pedigrees mapping to the dominant optic atrophy locus on 3q28-qter. *M. Votruba[1,2], D. Thiselton[1], A.T. Moore[2], S.S. Bhattacharya[1], B. Kjer[3], T. Rosenberg[3], H. Eiberg[4]*. 1) Dept of Molecular Genetics, Institute of Ophthalmology, London, UK; 2) Moorfields Eye Hospital, London, UK; 3) National Eye Clinic, Hellerup, Denmark; 4) Dept of Medical Biochemistry and Genetics, The Panum Institute, University of Copenhagen, Denmark.

Dominant optic atrophy (DOA, gene-*OPA1*), a hereditary optic neuropathy causing decreased visual acuity, colour vision deficits, a centro-caecal scotoma and optic nerve pallor, has been mapped to a genetic interval of 1.4 cM between loci D3S3669 and D3S3562 on chromosome 3q28-qter. In order to further refine the critical disease interval, and to test the power of haplotype analysis and linkage disequilibrium mapping, we identified a total of 48 families with DOA, unrelated on the basis of genealogy, from a database of genetic eye disease families. 38 families originated from the British Isles and 10 families from Denmark, and all showed evidence of linkage to the *OPA1* locus. They were genotyped with 9 highly polymorphic microsatellite markers spanning a region of 12 cM around the *OPA1* locus. Allelic frequency analysis [(Chi-squared test, Likely-hood Ratio Test (LRT) and p values)] and haplotype parsimony analysis showed evidence of a founder effect. Five markers (D3S1314, D3S3669, D3S1523, D3S2305 and D3S3562), spanning 4.4 cM across the disease-associated region, demonstrated significant linkage disequilibrium by LRT ($p<0.05$). A peak LRT of 31.64 ($p<0.00000001$, $l=0.64$), and a peak chi-sq of 37.06 ($p<0.00003$) occurred at D3S1523. From haplotype parsimony analysis of the Danish pedigrees alone, it would appear that 7 out of 10 of the Danish pedigrees are party to the founder effect. Analysis of the British pedigrees alone has previously indicated that 36 out of 38 pedigrees were likely to be ancestrally related. This new data suggests that the UK and the Danish populations with dominant optic atrophy may share a common core founder haplotype.

Program Nr: 2562 from the 1999 ASHG Annual Meeting

Hypomagnesemia with secondary hypocalcemia (HSH): Narrowing the Disease Region on Chromosome 9. *R.Y. Walder[1], Z. Borochowitz[4], H. Shalev[5], R. Carmi[5], K. Elbedour[5], D.A. Scott[2], E.M. Stone[3], V.C. Sheffield[2].* 1) Dept Internal Medicine, Univ Iowa, Iowa City, IA; 2) Dept Pediatrics; 3) Dept Ophthalmology; 4) Simon Winter Institute for Human Genetics, Haifa, Israel; 5) Genetics Institute, Soroka Medical Center, Ben Gurion University of Negev, Beer-Sheva, Israel.

Familial hypomagnesemia is an autosomal recessive disorder associated with secondary hypocalcemia (OMIM 602014). Affected individuals have extremely low levels of magnesium in the blood, and are frequently characterized by muscle weakness, tetany and seizures. Earlier work from our laboratory using DNA samples from three inbred Bedouin kindreds, containing 13 affected individuals, 11 males and 2 females, identified a region on chromosome 9 (9q12-9q22.2) linked to the disease. The linked interval spanned 14 cM and contained a large region of homozygosity, flanked by proximal marker D9S1874 and distal marker D9S1807. Several markers within this interval were linked with a LOD >3.0, with a maximum LOD of 7.14 for D9S284 at O= 0. Two unaffected individuals from the Bedouin kindreds showed recombinations within the HSH disease interval, narrowing the proximal flank to D9S1115. This narrowed genetic region is approximately 2.8 cM. In the present study, we describe two additional families, unrelated to the Bedouin kindreds, which are linked to the same interval on chromosome 9. There is no evidence of genetic heterogeneity in all of the HSH containing families that we have tested. One of these additional families contains two affected individuals with observed recombinations for D9S276 and D9S175. These recombinant individuals allow us to refine the interval to less than 1 cM, flanked by D9S1115 and D9S175. We have developed a YAC contig across the narrowest interval and using BACs and RH mapping, constructed a fine physical map of the HSH disease region. This map was used to search for ESTs in the interval. Selected BAC clones were sample sequenced to identify putative candidate genes from the region. This map will facilitate the positional cloning of the gene for HSH.
>q.

Program Nr: 2563 from the 1999 ASHG Annual Meeting

Genome scan for generalised nodal osteoarthritis susceptibility loci. *G.A. Wallis[1], R. Varhol[1], M.E. Grant[1], S. Eyre[1,2], P. Roby[1,2], W.O. Ollier[2], M. Doherty[3].* 1) Sch Biological Sci, Univ Manchester, Manchester, UK; 2) ARC ERU, Univ Manchester, Manchester, UK; 3) Dept Rheumatology, Univ Nottingham, Nottingham, UK.

Osteoarthritis (OA) is a heterogeneous disorder which represents the most common form of human joint disease. There is evidence that environmental and genetic factors contribute to the aetiology of the condition. Of the common subsets of idiopathic OA, generalised, nodal OA (GNOA) has the strongest familial predisposition and we therefore chose this form of OA for our search for OA susceptibility genes. For this purpose, families were recruited where there was at least one affected sibling pair (ASP) and the affected individuals had definite nodes on two or more distal interphalangeal joints of each hand plus radiographically confirmed OA in at least two sites (hands being one site). In a first stage genome scan, we have genotyped 249 individuals from 90 families including 187 affected females, 30 affected males and 163 ASP combinations using a standard set of microsatellite markers that span the genome. Analysis of the data using non-parametric pair wise linkage analysis identified 3 loci with a p less than 0.05: 2q24 (lod 1.45), 5cent (lod 0.8) and 8q24 (lod 1.4). Linkage between these loci and GNOA is currently being examined by inclusion of an additional 103 families (194 ASP combinations) and markers that span these loci in multipoint analyses.

Program Nr: 2564 from the 1999 ASHG Annual Meeting

construction of a physical and transcript map for a 1 Mb genomic region containing the urofacial (Ochoa) syndrome gene on 10q23-q24 and localization of the disease gene within two overlapping BAC clones. *C.Y. Wang, J.D. Sh1, Y.Q. Huang, P.E. Cruz, A. Davoodi-semiromi, J.X. She.* Dept Pathology/Immunol/Lab Med, Univ Florida, Gainesville, FL.

The urofacial (Ochoa) syndrome is an autosomal recessive disease characterized by distorted facial expression and urinary abnormalities. Previously, we mapped the UFS gene to chromosome 10q23-q24 and narrowed the interval to one YAC clone of 1,410kb. Here, we have constructed a BAC/PAC contig of the 1 Mb region using STS content mapping with 42 BAC/PAC-end sequences, 9 previously reported and 16 newly identified microsatellite markers and 14 EST markers. A total of 26 polymorphic microsatellite markers were genotyped for 31 UFS patients from Colombia and 2 patients from the US. Haplotype analyses suggest that the UFS gene is located within two overlapping BAC clones, a region of less than 360kb of DNA sequences. We tested 42 EST markers previously mapped to the D10S1709-D10S603 interval against the BAC/PAC contig and identified 11 ESTs located in the 1Mb region. Four of the 11 ESTs mapped to the 360kb UFS critical region. Shotgun sequencing of the two BAC clones and BLASTN search of the EST databases revealed three other ESTs contained in the UFS critical region. These results will facilitate the cloning and identification of the UFS gene.

Program Nr: 2565 from the 1999 ASHG Annual Meeting

Genome-Wide Linkage Analysis of Type 2 Diabetes-Related Quantitative Traits in the FUSION Study. *R.M. Watanabe[1], C.D. Langefeld[1], M. Epstein[1], T. Valle[1], S. Ghosh[1], F.S. Collins[1], R.N. Bergman[4], M. Boehnke[1], The FUSION Study Investigators[1,2,3,4].* 1) Dept. of Biostatistics, Univ. of Michigan School of Public Health, Ann Arbor, MI; 2) National Public Health Institutes, Helsinki, Finland; 3) National Human Genome Research Insititute, Bethesda, MD; 4) Dept. of Physiology & Biophysics, Univ. of Southern California, Los Angeles, CA.

The FUSION study is an effort to positionally clone genes for type 2 diabetes and related quantitative traits in a Finnish cohort. We sampled 580 families with type 2 diabetes (1240 affected subjects, 2095 total subjects) ascertained through affected sibling pairs, and have genotyped these subjects for >400 genetic markers spaced ~10 cM apart throughout the genome. Quantitative trait locus (QTL) linkage analysis was performed using variance components (VC) analysis of intact families using the program USERFQTL which combines elements of SIBLINK for identity-by-descent estimation in relative pairs and FISHER for VC estimation. We analyzed body mass index (BMI), waist-to-hip ratio (WHR), fasting and 2-hour glucose (GLU) and insulin (INS), fasting C-peptide, minimal model-derived measures of glucose effectiveness and insulin resistance, and acute insulin response (AIR) after adjusting for age and gender. Analyses were also done with and without additional adjustment for BMI or WHR, due to the known relationship between adiposity and type 2 diabetes. Affected (AFF) and unaffected (UAF) individuals were analyzed independently, because disease status may differentially affect trait values. Also, unaffected offspring (OFF) were analyzed as an additional "at-risk" group. Our most interesting results to date include: AIR on chr. 10 (UAF, LOD=3.11, distance from pter d=21 cM), fasting INS on chr. 14 (AFF, LOD=2.67, d=102 cM), fasting GLU on chr. 14 (OFF, LOD=2.66, d=23 cM), 2-hour GLU on chr. 20 (OFF, LOD=2.11, d=9.5 cM). These initial results provide evidence for several quantitative trait loci along the genome. In addition, because these traits are closely related to type 2 diabetes, these QTL linkage results may provide information regarding the location of type 2 diabetes susceptibility loci.

Program Nr: 2566 from the 1999 ASHG Annual Meeting

High throughput whole genome polymorphism scans. *J.L. Weber, W. Dickinson, T.A. Kronenwetter-Koepel, L. Ott, T.L. Rusch, D.A. Vaske, C. Zhao.* Center for Medical Genetics, Marshfield Medical Research Foundation, Marshfield, WI.

Human whole genome polymorphism scans are currently used widely in linkage mapping and in the future will also likely find broad clinical application. While diallelic polymorphisms have received a great deal of attention and hold great potential, no one has yet been able to devise an effective means of employing diallelic polymorphisms in whole genome scans. Fortunately, genotyping of multiallelic short tandem repeat polymorphisms (STRPs) continues to steadily improve. In 1999, whole genome scans using about 400 STRPs will be completed at Marshfield on over 13,000 individuals at a total cost, including indirect costs, of about $150 per individual (< $0.40 per genotype). Genotyping quality has remained fairly constant during productivity improvement. Genotyping completeness averages about 97.5%; and genotyping accuracy about 99.5%. Primary sources of cost reductions have been experience of laboratory members, use of optimal markers, economies of scale, and, most importantly, improvements in technology. Major and minor improvements have been made in the laboratory processes for genotyping, in the instruments used in the genotyping process, and in the software and computer systems for allele calling and data management. Our large capacity water bath thermal cyclers have proven highly reliable and cost effective. Our Scanning FlUorescent Detectors (SCAFUDs) currently utilize 200 lane gels and 16 channel PMT array detectors, so that up to 7 different visible fluorescent dyes and 33,600 genotypes can be obtained in each gel run. Excluding dropouts, our allele calling software currently has an accuracy of about 95%. A new screening set of 400 STRPs (Screening Set 10), recently developed by D. Vaske, eliminates markers which were particularly difficult to score, and provides improved spacing of markers along the chromosomes and better coverage of telomeres. Many additional improvements in STRP genotyping are in progress. Within two-three years it is likely that 400 marker total genotyping scan costs can be reduced to to $100 or less per individual ($0.25 per genotype). Plans are also in place to steadily expand our marker screening set to 800-1,000 STRPs.

Program Nr: 2567 from the 1999 ASHG Annual Meeting

Refinement of the Limb Girdle Muscular Dystrophy Type 2H (LGMD2H) Candidate Region. *T. Weiler[1], T. Sudha[1], E. Nylen[1], T.M. Fujiwara[2], K. Morgan[2], C.R. Greenberg[1], K. Wrogemann[1]*. 1) Univ. Manitoba, Winnipeg, MB, Canada; 2) McGill Univ., Montreal, PQ, Canada.

Limb girdle muscular dystrophy type 2H (LGMD2H) is an autosomal recessive disorder initially described by Shokeir and Kobrinsky in 1976 in Manitoba Hutterites (Clin. Genet. 9:197-202, 1976). We have recently mapped the location of this gene to chromosome 9q31-q33 in a 4.4 Mbp region (Genetic Location Database, LDB), flanked by *D9S302* and *D9S1850* (AJHG 63:140-147, 1998). Here we report the identification of additional Manitoba Hutterite families affected with LGMD2H, the refinement of the *LGMD2H* candidate interval, extensive re-ordering of the region on the basis of YAC contigs, genomic sequences, radiation hybrid (RH) mapping and recombination events in LGMD2H & CEPH families.

Two new nuclear families with 3 LGMD patients have been identified and analyzed. Two of these patients are in their late 50's with a history of elevated creatine kinase (CK) levels. Both are now wheelchair bound. The other patient is 40 yrs old, exhibits proximal weakness and has an elevated CK level. These two families as well as the four previously reported families have been genotyped with 17 additional markers located between *D9S302* and *D9S1850*. Analysis of haplotypes revealed recombination events in this region in five individuals refining the *LGMD2H* candidate region to an area flanked by *D9S1855* and *D9S1802*. The order of the markers in this region however, based on the LDB, Marshfield and GeneMap '99 maps and 1 Mbp of genomic sequence, is not in agreement. Hence, haplotypes of CEPH individuals and a YAC contig were constructed and RH mapping using the TNG panel from the Stanford Human Genome Centre (SHGC) was performed. The high resolution TNG panel has been screened for all the markers in the candidate region. Together, the 6 recombinant chromosomes, the YAC contig, the 9q32 genomic sequence and the RH map of the *LGMD2H* region have served to order the markers and have reduced the size of the candidate area.

Program Nr: 2568 from the 1999 ASHG Annual Meeting

Linkage Mapping Set-HD5 on ABI Prism® 377, 310 and 3700 DNA Analyzers. *A. Wheaton, K. Rogers, M. Roque-Biewer, Y. Wang, M. Rivera, N. Caffo, P. Dong.* Genetic Analysis R&D, PE Biosystems, Foster City, CA.

The newly introduced Linkage Mapping Set-HD5 (LMS-HD5) is a set of optimized microsatellite markers originally identified in the Genethon Human Linkage Map (Dib et al. 1996 Nature 380:A1-138), that provides an average 5 cM genome-wide coverage. LMS-HD5 utilizes the dye set 6-FAM, HEX, NED, and ROX, and the reverse primers are tailed on the 5 end to minimize plus A inconsistencies. The set incorporates the 400 markers originally in the Linkage Mapping Set-MD10 (previously LMS V2) and 411 newly designed markers, for a total of 811 markers. We will demonstrate the cross-platform performance of the Linkage Mapping Set -HD5 on the ABI Prism 377, 310 DNA sequencers, and the ABI Prism 3700 DNA analyzer. We will describe optimized pooling ratios of PCR products for each platform as well as demonstrate sizing accuracy across platforms. As expected sizing estimates of the same DNA fragment can vary across instrument platforms. We will show that the sizing precision is within a +/- 0.15bp standard deviation on all instrument platforms.

Program Nr: 2569 from the 1999 ASHG Annual Meeting

The insulin receptor related receptor gene (*IRR*) is mapped to chromosome 1q21-q23: A candidate diabetes susceptibility locus. *T.E. Whitmore, M.F. Maurer, H.L. Day, A.C. Jelmberg, M.M. Dasovich, S.K. Burkhead, M.D. Heipel, S.R. Jaspers, R.D. Holly, S. Lok.* ZymoGenetics Inc., Seattle, WA.

The insulin receptor related receptor (IRR) is an orphan member of the insulin receptor family. The receptor is a heterotetrameric glycoprotein consisting of extracellullar alpha-subunits comprising the ligand binding domain that are disulfide-bonded to beta-subunits spanning the cell membrane and contain a cytoplasmic tyrosine kinase which is activated upon ligand binding. *IRR* is expressed in sensory and sympathetic neurons, renal epithelial cells, enterochromaffin-like cells of the fundic stomach and islet beta cells where it has been proposed to regulate the physiological function of insulin secreting cells (Ozaki 1998, Eur. J. Endocrinol. 139: 244). While a ligand for IRR has not been identified, the IRR signaling pathway includes the phosphorylation of insulin receptor substrate-2 (Hirayama et al. 1999, Diabetes 48: 1237), a cytosolic docking protein believed to regulate compensatory increase in pancreatic beta-cell mass (Withers et al. 1998, Nature 391: 900). As a first step to assess a possible role of *IRR* in heritable forms of diabetes, we carried out a regional mapping of the human *IRR* locus. The *IRR* locus was mapped to chromosome 1 using the Stanford G3 Radiation Hybrid Mapping Panel. The results showed linkage of *IRR* to chromosome 1 marker *SHGC-69054* with a LOD score of 11.39 and a distance of 0 cR from the marker. Comparison with the surrounding cytogenetically mapped genes positions *IRR* in the 1q21-q23 region of chromosome 1. The *IRR* locus is approximately 1.5 Mb proximal to *APOA2*, in a region that is implicated for a major recessive diabetes locus identified from a new genome-wide search of susceptibility genes in multigenerational families of Northern European ancestry (Elbein et al. 1999, Diabetes 48: 1175). Additionally, a genomic scan for loci linked to type II diabetes and body-mass index in Pima Indians has also indicated a potential diabetes susceptibility locus in the 1q21-q23 region (Hanson et al. 1998, Am. J. Hum. Genet. 63: 1130). These results support *IRR* as a strong candidate for a diabetes susceptibility gene locus residing on chromosome 1q21-q23.

Program Nr: 2570 from the 1999 ASHG Annual Meeting

Genomic screen for primary open angle glaucoma susceptibility genes. *J.L. Wiggs[1], J.L. Haines[2], A.M. Hossain[2], J. Kern[1], E.A. del Bono[1], B. Broomer[3], F.L. Graham[3], M.A. Pericak-Vance[3], R.R. Allingham[3].* 1) Depts of Ophthalmology and Genetics, New England Medical Ctr, Boston, MA; 2) Dept of Genetics, Vanderbilt University, Nashville, TN; 3) Depts of Ophthalmology and Genetics, Duke University, Durham, NC.

Primary open angle glaucoma (POAG) is the leading cause of irreversible blindness worldwide. POAG is characterized by progressive optic nerve atrophy, visual field loss, and often elevated intraocular pressure. Onset is usually after the fifth decade. A family history of POAG is a significant risk factor for the disease and indicates a genetic etiology. Previous studies have suggested that multiple genetic and possibly environmental factors may participate in the disease process. To locate genes that may be associated with POAG, we have completed an initial genome screen using 103 affected sibpairs (121 affected individuals). POAG was defined as: age of diagnosis greater than 35, IOP greater than 22mmHg 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 were excluded. Four hundred and forty-five markers spanning the human genome at approximately 7 cM intervals were analyzed. Linkage analysis was performed using both model-dependent (lod score) and model-independent affected relative pair, and sibpair methods. Sixteen regions on chromosomes 1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 14, 15, 17, and 19 showed interesting results (p-value <0.05 and/or a lod score >1.0). Using a second pedigree set of 69 affected sibpairs (81 affected individuals) continued positive results have been found for four of these regions (chromosomes 2, 9, 14 and 19). The region identified on chromosome 2 may correspond to a previously identified glaucoma susceptibility locus (GLC1B), while the other regions are novel. Further follow-up studies are in progress.

Program Nr: 2571 from the 1999 ASHG Annual Meeting

Chromosome 12 and late onset Alzheimer's disease. *E.M. Wijsman[1], E.W. Daw[1], E.J. Nemens[2], H. Payami[3], T.D. Bird[2], G.D. Schellenberg[2]*. 1) Dept Medical Genetics, Univ Washington Sch Medicine, Seattle, WA; 2) VA Medical Center, Seattle, WA; 3) Oregon Health Sciences University, Portland, OR.

While 3 genes have been identified that play a role in early onset Alzheimer's disease (AD) only ApoE has been definitively associated with late-onset AD (LOAD). Recently, reports of linkage and combined linkage and association studies have implicated genes on chromosome 12, including the region between D12S273 and D12S390, and the genes LRP and A2M. These await confirmation.

We have performed analyses of 67 LOAD families ranging in size from 4 to 53 individuals (685 total individuals) with 23 chromosome 12 markers, plus LRP and A2M. ApoE was included as a major-gene covariate. The method of analysis was Monte Carlo Markov chain joint oligogenic segregation and linkage analysis which gives accurate localization even in the presence of genetic heterogeneity. Observed age-of-onset or censoring was used as the phenotype. Covariate effects were estimated as shifts in age-of-onset. Evidence for linkage was assessed by the ratio of observed to expected placements of trait loci. We found modest evidence for linkage (~4 fold increase in trait locus placements over baseline) at about 38cM on the Marshfield map. We found no evidence for linkage between D12S273 and D12S390. In a joint major gene covariate analysis of LRP, A2M and ApoE, ApoE genotype effects were as previously reported, but there was no evidence that LRP genotypes differed in age-of-onset (mean difference, d, between the heterozygote and common homozygote of d=0.0033 yrs, sd=1.64 yrs). For A2M, there was a modest decrease in age-of-onset for the heterozygote relative to the most common homozygote (d=.85 yrs, sd=1.62 yrs) and no difference between the heterozygote and rare homozygote. The common allele was reported previously to be associated with increased AD risk, while here the risk associated with this allele decreased. Our results provide little or no evidence supporting the role of LRP and A2M in AD risk, exclude linkage from one region of chromosome 12, and provide at best weak evidence for linkage to the other region. Supported by NIH AG05136 and GM46255.

Program Nr: 2572 from the 1999 ASHG Annual Meeting

A genome-wide scan in 72 sib-pair families with schizophrenia reveals suggestive evidence for 7 potential susceptibility loci. *D.B. Wildenauer[1], S.G. Schwab[1], J. Hallmayer[2], B. Lerer[3], K. Kanyas[3], M. Albus[4], G.N. Eckstein[1], M. Borrmann[4], M. Rietschel[1], D. Lichtermann[1], M. Trixler[5], W. Maier[1]*. 1) Dept Psych, Univ Bonn, Germany; 2) Graylands Hospital/University of Western Australia, Mt Claremont, Australia; 3) Dept Psych, Hadassah University Medical Center, Jerusalem, Israel; 4) Mental State Hospital, Haar, Germany; 5) Dept Psych, University Medical School of Pecs, Hungary.

Schizophrenia is a devastating mental disorder which affects worldwide up to 1% of the population. A role of genetic factors in development of the illness is documented by family-, twin-, and adoption studies. These studies and the failure to detect Mendelian inheritance by segregation analysis suggests polygenic inheritance and environmental influences. We have ascertained 72 families (470 individuals) through a schizophrenic or chronic schizoaffective (RDC) index patient, each family having at least 2 affected siblings and parental information (67 families with both parents, 5 with one parent and unaffected siblings). 13 families were from Israel, 59 from Germany. The sample comprises a total of 87 (n-1) and 107 (n(n-1)1/2) sib-pair combinations for affected sib-pair analysis, identity by descent. 415 fluorescence labeled microsatellite marker have been used for genotyping in approximately 10 cM average distance. In addition to previously in our sample detected potential susceptibility loci on 6p22,23, 10p14-11, 22q12,13 (supported by other groups in independent samples),18p11.2 (supported by linkage disequilibrium and by overlapping with findings in bipolar disorder), suggestive evidence was obtained for 4q (NPL=2.7, p=0.003 around D4S2917) and 6q (NPL=2.4, p=0.008 around marker D6S1613). A potential locus on 5q31 (around D5S399) which had been previously detected in a subsample of 44 families was not supported in the genome scan sample of 72 families (mlod = 0.64 for D5S399). However, 25 cM telomeric to D5S399, a mlod of 1.68 (NPL=2.5, p=0.006) was obtained for marker D5S422. The study demonstrates difficulties in mapping susceptibility loci for schizophrenia, but provides also suggestive evidence for regions which may be screened for candidate genes.

Program Nr: 2573 from the 1999 ASHG Annual Meeting

Confirmation of linkage between the HLA-DR locus and multiple sclerosis in two unusually large MS families.

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Multiple sclerosis (MS) is a complex trait. Genetic analysis of MS has typically been approached with an affected relative pair analysis. Complexity seems certain and even the large numbers of sibling pairs available may not find all susceptibility genes by linkage methods. Previous association studies have implicated the human leukocyte antigen (HLA) genes within the major histocompatibility complex (MHC) on 6p21 as being involved in MS susceptibility. Four recent genome scans using sibling pairs have demonstrated the involvement of the MHC region in MS susceptibility. However, evidence for other loci remains modest. One approach to the problem of complexity is to examine isolated pedigrees with a high recurrence rate.

The Canadian Collaborative Project on the Genetic Susceptibility to Multiple Sclerosis (CCPGSMS) has ascertained over 15,000 individuals with MS. The CCPGSMS has identified over 1,000 families with at least two affected individuals and blood samples from 900 families have been collected. 110 Pedigrees with more than four affected family members were chosen for possible genetic study. The two largest families, with 14 and 9 affected individuals respectively, detected linkage to the HLA-DR locus with a lod score of 2. This supports the suggestion based on clinical findings that these families have a type of MS in which the MHC plays a similar role in susceptibility to that seen in families with one or two affected individuals.

Future studies of multiple sclerosis may be aided by focusing on large kindreds to confirm or identify regions of susceptibility to MS. Aside from the labour and cost advantages, large kindreds are more likely to be homogeneous than populations of affected relative pairs.

Program Nr: 2574 from the 1999 ASHG Annual Meeting

Linkage of a Gene Causing Familial Focal Segmental Glomerulosclerosis. *M.P. Winn[1], P.J. Conlon[2], K.L. Lynn[3], D.N. Howell[4], B.D. Slotterbeck[1,5], A.H. Smith[3], F.L. Graham[1,5], M.L. Bembe[1,5], L.D. Quarles[1], M.A. Pericak-Vance[1,5], J.M. Vance[1,5]*. 1) Medicine, Duke University Medical Center, Durham, NC; 2) Nephrology, Beaumont Hospital, Dublin, Ireland; 3) Nephrology, Christchurch Hospital, New Zealand; 4) Pathology, Duke University Medical Center, Durham, NC; 5) Center for Human Genetics, Duke University Medical Center, Durham, NC.

Focal segmental glomerulosclerosis (FSGS) is a pathological entity characterized by proteinuria, nephrotic syndrome and the progressive loss of renal function. It is a common cause of end-stage renal disease (ESRD). Autosomal dominant and recessive forms of FSGS have been identified as well as types associated with congenital syndromes. Two families with autosomal dominant FSGS were evaluated for linkage using 351 genomic microsatellite markers. Linkage, multipoint analysis, and tests for heterogeneity were performed on the subsequent results. In addition, three small families were utilized for haplotype analysis. Evidence for linkage was found on chromosome 11q21-22 for the largest family, with a maximum LOD score of 9.89. The gene is currently localized to an approximately 8 cM area between flanking markers D11S919 and D11S1986. The disease in a second family was not linked to this locus, or to a previously described locus on chromosome 19q13. There were no shared haplotypes among affected individuals in the three smaller families. Our findings demonstrate that genetic heterogeneity is prevalent in FSGS in that at least three genes cause the FSGS phenotype. Identification of the genes which cause familial FSGS will provide valuable insights into the molecular basis and pathophysiology of FSGS.

Program Nr: 2575 from the 1999 ASHG Annual Meeting

Linkage analysis of candidate regions in Swedish non-syndromic cleft lip and palate families. *F.K. Wong[1,2], C. Hagberg[2], A. Karsten[2], O. Larson[3], J. Huggare[2], C. Larsson[1], B.T. Teh[1], S. Linder-Aronson[2].* 1) Molecular Medicine, Karolinska Hospital , Stockholm, Sweden; 2) Orthodontics, Karolinska Institutet, Stockholm, Sweden; 3) Reconstructive Plastic Surgery, Karolinska Hospital, Stockholm, Sweden.

Objective: To analyze linkage of four candidate regions for non-syndromic cleft lip and palate (CLP) on chromosome 2p13, 4q, 6p23 and 19q13; in addition chromosome 1q32, the locus for van der Woude syndrome (VWS), on Swedish CLP families. Design: Three to five linked microsatellite markers were selected from each candidate region. Polymerase chain reaction (PCR) with fluorescent labeled markers was performed on DNA samples from the participating families. Electrophoresis of the PCR products was performed on a laser-fluorescent DNA sequencer. The genotype data was analyzed with multi-point linkage analysis. Mode of inheritance tested included two autosomal dominant, an autosomal recessive and a non-parametric model. Multi-point LOD scores were also calculated by assuming genetic heterogeneity. Participants: Nineteen Swedish families each with at least two members affected with CLP. Results: Cumulative multi-point LOD scores calculated under autosomal dominant modes of inheritance were less than -2 except chromosome 6p23. LOD scores calculated under recesive inheritance and the non-parametric model were inconclusive. There was no significant evidence of genetic heterogeneity among the sample group. Conclusions: Linkage of the Swedish CLP families to the candidate regions (1q32, 2p13, 4q, 6p23 and 19q13) could not be established. This may suggest a new but yet unknown CLP locus or loci in the Swedish families. The results may, however, reflect the heterogeneity of CLP which is difficult to confirm statistically in this sample group. The families collected and the linkage results from this study will form the base for future genetic studies of CLP in this population.

Program Nr: 2576 from the 1999 ASHG Annual Meeting

Mapping of Familial Partial Epilepsy with Variable Foci to 22q12 and Genotype-Phenotype Correlation. *L. Xiong[1,2], M. Labuda[1], J.C. Mulley[5], D.S. Li[1], R. Desbiens[3], S. Verret[3], P. Langevin[3], S. Mercho[2], M.H. Seni[2], F. Dubeau[2], S.F. Berkovic[1,4], J. Serrotosa[6], F. Andermann[2], E. Andermann[2], M. Pandolfo[1,2].* 1) Notre Dame Hospital, CHUM, Montreal, Quebec, Canada; 2) Dept Neurology & Neurosurgery, Montreal Neurological Inst, Montreal, PQ, Canada; 3) CHUQ, Quebec, Quebec, Canada; 4) Austin and Repatriation Medical Centre, Melbourne, Australia; 5) Women's and Children's Hospital, North Adelaide, Australia; 6) Neurology Service, Fundacion Jimenez, Madrid, Spain.

We previously described three large French Canadian families presenting with a clinically homogeneous phenotype of familial partial epilepsy with variable foci (FPEVF). Genotyping, using polymorphic microsatellite markers and linkage analysis by maximum-likelihood parametric approach and a dominant model with 50% penetrance was carried out. Candidate loci were tested and a whole genome scan was performed. We identified linked markers to chromosome 22q12 for the two largst families with a combined lod score of 6.58. Another French Canadian family with 7 affected individuals presenting with the same phenotype was subsequently identified showing linkage to the same locus and sharing the same haplotype. By analysis of recombination events, the disease locus was localized within a 3.7 cM interval. A Spanish family with a similar phenotype also showed evidence of linkage (lod score=2.8), but with a different haplotype. Genotype-phenotype correlation revealed that the phenotypic spectrum within the three families includes partial seizures arising from frontal, temporal or occipital foci in different affected individuals, with age of onset varying from 3 to 25 years. Individuals with symptomatic epilepsy, isolated deja vu symptoms, other episodic disorders were not found to carry the disease haplotype. One of the probands was found to be homozygous for the linked haplotype. None of 21 additional families presenting with various familial partial epilepsies showed linkage to the same locus.Mutation analysis excluded a few candidate genes within the region, including 14-3-3 protein, RRP22 protein, etc.. Our results support the view that familial partial epilepsy can be caused by syndrome-specific genes with Mendelian inheritance.

Program Nr: 2577 from the 1999 ASHG Annual Meeting

Determination of SNP Allele Frequency by A DNA Pooling Method. *C. Xu, C. Donnelly, D. Montgomery, C. Allan, I. Purvis.* Dept Molecular Genetics, Glaxo Wellcome R&D, Hertfordshire, England.

Allelic association study is one of the most powerful methods for mapping genes that contribute to complex diseases. However, due to the large number of markers and DNA samples required, it is very labour-intensive and expensive to carry out such studies. DNA pooling has been shown to be an effective preliminary screen method for allelic association studies, which can reduce the labour and cost significantly. We described a TaqMan method to determine the allele frequencies of single nucleotide polymorphisms by DNA pooling. The allele frequencies of 3 SNPs were determined in a pool consisting of 80 individuals. These estimated allele frequencies by DNA pooling were then compared to that calculated from the genotype of each individual. Accurate estimation was also obtained even at very low concentrations of DNA. In addition, this method detected small increases or decreases in allele frequencies of SNPs within the pool indicating that it is a feasible way to determine allele frequencies in association studies. Our results showed that Taqman is an effective and efficient method in determining SNP allele frequencies in a DNA pool.

Program Nr: 2578 from the 1999 ASHG Annual Meeting

A genome-wide search for Crohn's disease susceptibility loci in Ashkenazi Jewish families pooled from four U.S. IBD research groups. *H. Yang[1], J.H. Cho[2], R.H. Duerr[3], K.D. Taylor[1], Y.C. Lin[1], J.I. Rotter[1], S.R. Brant[4].* 1) Div. Med Genetics, Cedars-Sinai Medical Ctr, Los Angeles, CA; 2) Univ. of Chicago, Chicago, IL; 3) Univ. of Pittsburgh, Pittsburgh, PA; 4) Johns Hopkins Univ., Baltimore, MD.

Background/Aims: Chronic inflammatory bowel disease (IBD) presents as two major clinical forms, Crohn's disease (CD) and ulcerative colitis (UC). The Ashkenazi Jewish population has a 3-4-fold greater risk for IBD than non-Jewish Caucasians. The estimated relative risk to siblings is much greater for CD (30) than that for UC (15). The aim of this study is to identify specific susceptibility loci for CD in Ashkenazi Jewish families with a genome-wide search approach. **Methods:** A total of 63 CD only families (13 extended and 50 sib-pair) from four IBD research groups were studied. The genotyping in these families was part of a whole genome scan effort in each group with different marker panels (ABI linkage panel 1 and 2, Weber screening set 8.0). To perform linkage analysis in the combined sample, we integrated all markers by using the Broman et al map (1998). Approximately 350 microsatellite markers with an average interval of 10 cM were used by each group. Multipoint linkage analysis was performed using GENEHUNTER2.0 and GENEHUNTER- PLUS1.1. **Results:** For the combined screen, evidence for linkage was strongest at chr 16p12 (multipoint logarithm of the odds, MLod=2.0, NPL=2.4, p=0.008), 30cM p-telomeric to the previously reported *IBD1* region. In addition, nominal evidence for linkage was observed at chr. 2p, 3q, 4q, 5q, and 17qter (Mlod>1.2, NPL>2, p<0.02). The 3q and 4q peaks overlie the same regions reported previously by Cho et al. (1998). No evidence was present for the *IBD2* locus on chr. 12 reported by Satsangi et al. (1996). **Conclusion:** These results provide evidence for potential susceptibility loci contributing to the risk of CD specifically in Ashkenazi Jewish families. *IBD1* may be more p-telomeric relative to previous reports or there is a 2nd chr 16 susceptibility locus. Further fine mapping is required to narrow the region and identify positional candidate genes which may be relevant to the pathogenesis of CD.

Program Nr: 2579 from the 1999 ASHG Annual Meeting

Sequence-based polymorphisms in members of the apoptosis Bcl-2 gene family and their association with hematocrit level. *J. Yankowitz[1], S. Zeng[1], J.C. Murray[2], J.A. Widness[2], R.G. Strauss[3]*. 1) Dept OB/GYN; 2) Dept Peds; 3) Dept Path, Univ Iowa Hosp & Clinics, Iowa City, IA.

Apoptosis, or programmed cell death, is an active process which controls cell number and quality. Apoptosis may play a role in hematopoiesis. The Bcl-2 gene family includes anti-apoptotic (bcl-2, bcl-xL, bclw) and pro-apoptotic (bcl-xS, Bak, Bax) genes. Members of the Bcl-2 family have been shown to mediate survival of erythroid cells. Deregulation of the Bcl-2 family may lead to an increase in red cell destruction and anemia. We sought to find polymorphisms in the bcl-x, Bax and bclw genes and determine whether they were linked to hematocrit.

We screened the 5'- and 3'-regions of bcl-x, Bax, and bclw using PCR and SSCP analysis. One sequence-based polymorphic locus was found in each 5'-region of Bax and bclw having 3 and 2 alleles respectively. No polymorphism was found for bcl-x. Strong linkage between the Bax polymorphism and several markers on chromosome 19 was seen. The bclw polymorphism was strongly linked to D14S50. Direct sequencing showed the Bax polymorphism to be caused by variation in adenine nucleotide repeat number (19, 25, 27) starting at position 360 and the bclw polymorphism is the result of a G to A transition at 550. The allelic frequencies for Bax were, 0.2 for A1, 0.08 for A2, 0.78 for A3, and for bclw 0.24 A and 0.76 for G. Both are in are in Hardy-Weinberg equilibrium. Using these Bax and bclw polymorphic markers, we genotyped 200 subjects with high or low hematocrit (Hct) selected from a population of 819 healthy Iowans. A comparison of the allelic frequencies and distribution of each polymorphism was made in the low vs high Hct, male vs female, male low vs male high Hct, female low vs female high Hct, male high vs female high Hct and male low vs female low Hct group. Statistical analysis indicated that the allelic frequencies of the polymorphic loci were not significantly different in any of the comparisons ($P>0.05$). Nucleotide variation at the 5-region of Bax or Bcl-w does not appear to be related to human Hct level. These polymorphisms can be a powerful tool to study other events which may be linked to the Bax and bclw genes.

Program Nr: 2580 from the 1999 ASHG Annual Meeting

Further refinement of the MYP2 locus for autosomal dominant high myopia by linkage disequilibrium analysis.
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High myopia (> -6.00 diopters) is a complex common disorder that predisposes individuals to retinal detachment, glaucoma, macular degeneration, and premature cataracts. Recent linkage analysis of 7 families with autosomal dominant high myopia has identified one locus (MYP2) for this disorder on chromosome 18p11.31 (AJHG 63:109;1998). Haplotype analysis revealed an initial interval of 7.6 cM.

We performed transmission disequilibrium testing (TDT) using both the S.A.G.E. 3.1 TDTEX and GENEHUNTER 2 programs of chromosome 18p marker alleles. The following p values were obtained for these markers in marker order used for fine mapping in this region; D18S1146 (p = 0.227), D18S481 (p = 0.001), D18S63 (p = 0.062), D18S1138 (p = 0.0004), D18S52 (p = 1.79 x 10-6), and D18S62 (p = 0.141) by S.A.G.E. analysis. GENEHUNTER 2 TDT analysis revealed the p values for the linked allele for the following markers; D18S1146 (allele 2, p = 0.083), D18S481 (allele 1, p = 0.108), D18S63 (allele 11, p = 0.034), D18S1138 (allele 6, p = 0.011), D18S52 (allele 4, p = 0.007), and D18S62 (allele 3, p = 0.479).

The data from both tests suggest that the gene for 18p11.31 linked high myopia is most proximal to marker D18S52, with a likely interval of 0.8 cM between markers D18S63 and D18S52. Due to the contraction of the interval size by TDT testing, it is now possible to begin physical mapping and gene identification in the MYP2 locus.

Acknowledgements: NIH grant 1K23-EY00376-01A1, and Research to Prevent Blindness, Inc.

Program Nr: 2581 from the 1999 ASHG Annual Meeting

Homozygosity and Linkage-Disequilibrium Mapping of Bardet-Biedl Syndrome Type 1 (BBS1). *T. Young[1], M.O. Woods[1], P.S. Parfrey[1], J.S. Green[1], D. Hefferton[1], W.S. Davidson[2].* 1) Fac Medicine, Memorial Univ Newfoundland, St John's, NF, Canada; 2) Dept Biochem, Memorial Univ Newfoundland, St John's, NF, Canada.

Bardet-Biedl syndrome (BBS; OMIM 209900) is a rare, autosomal recessive disorder of unknown etiology that exhibits phenotypic and genetic heterogeneity. The syndrome is characterized by retinal dystrophy, dysmorphic extremities, obesity, male hypogenitalism and renal anomalies. Five genetic loci have been mapped by genome-wide scanning in (i) a collection of North American families of North European descent (BBS1, 11q); (ii) three unrelated Bedouin kindreds from Kuwait (BBS2, 16q; BBS3, 3p; BBS4, 15q); (iii) a kindred of North European descent from Newfoundland, Canada (BBS5, 2q). So far, none of the BBS genes have been cloned. We have extended haplotypes within the 13 cM BBS1 critical region for 10 BBS patients and their families representing three BBS1-linked and three unassigned families from the recently founded Newfoundland population. All 10 patients were homozygous for overlapping portions of a rare, disease-associated ancestral haplotype. Linkage disequilibrium analysis of the overlapping ancestral haplotypes significantly reduced the BBS1 critical region and should aid in the positional cloning of the BBS1 gene.

Program Nr: 2582 from the 1999 ASHG Annual Meeting

Evidence for Linkage to 10q21-22 for Mitral Valve Prolapse Phenotype in a Dilated Cardiomyopathy Family. *B. Yuan[1], S.H. Duan[1], G. Schonfeld[1], Y. Von Kodolitsch[2], M. Bourdius[2], M. Malkowski[2], R.E. Pyeritz[2].* 1) Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO, USA; 2) Department of Human Genetics, MCP Hahnemann School of Medicine, Pittsburgh, PA, USA.

Mitral valve prolapse (MVP) is the most common form of valvular defect with a prevalence of 4-6%. Idiopathic dilated cardiomyopathy (DCM) is the most common form of heart disease with Mendelian inheritance. We evaluated a family showing autosomal dominant transmission of DCM associated with MVP through at least 3 generations (7 affected). Echocardiography in the youngest 2 generations showed MVP in all 3 instances of DCM; however, 4 young individuals with normal left ventricular dimensions and function had MVP; 3 also had mild mitral regurgitation (MR). None had signs of a systemic disorder of connective tissue, conduction abnormality or rhythm disturbance. Genetic mapping using polymorphic markers around previously reported loci for familial DCM showed a positive LOD score only for markers at 10q21-22. The 2-point LOD for D10S556 was 3.3 (q = 0; penetrance = 0.99). Multipoint non-parametric analyses (GENEHUNTER v1.2) showed a maximal peak of NPL=6.27 (p=0.005) in the 6 cM region bounded by D10S1650 and D10S607. MVP phenotype was used in both of the analysis. There was no linkage detected to this locus with DCM phenotype. Analysis showed that MVP in this family segregated with a distinct haplotype. In another family, DCM associated with MVP was previously mapped to the same region. Our results suggest, first, that in some forms of DCM, MVP is an early manifestation of the basic defect, and second, that a locus exists at 10q21-22 that encodes a product that is essential for long-term myocardial function. While MVP and MR can be a consequence of left ventricular dilatation, dysfunction, or both, our family suggests that at least one structural abnormality of the MV apparatus is a primary component of this DCM phenotype. A variety of genes encoding cytoskeletal proteins map to the region and a search for mutations will ensue.

Program Nr: 2583 from the 1999 ASHG Annual Meeting

On a Unified Transmission/Disequilibrium Test. *H. Zhao[1], F. Sun[2]*. 1) Epidemiology & Public Health, Yale Univ Sch Medicine, New Haven, CT; 2) Department of Genetics, Emory UNiversity, Atlanta, GA.

The transmission/disequilibrium test (TDT), which is based on the unequal probability of transmission of different marker alleles from parents to the affected offspring, may have greater power to detect linkage for complex traits than traditional family-based linkage methods. Since the original TDT was proposed for studying families with both parents available, the TDT has been extended to analyzing sibships without parents and one affected child with only one parent. However, these extensions are still restricted to limited family structures. For example, these methods cannot analyze the following two cases: (1) a set of affected siblings without parents; and (2) multiple sibs with one available parent. Because the real data do not necessarily fall into the types studied so far in the literature, there is a real need to develop a unified approach to analyzing families of different structures. We have recently developed a likelihood-based approach to studying transmission disequilibrium that simultaneously uses nuclear families of different structures. This approach borrows information across all the families to infer mating types for each pair of parents (genotypes available or not). As a result, this approach extracts more genetic information from family data and is more efficient than existing methods. Simulation studies under different population structures and disease models also demonstrated the greater power of the proposed method. For example, in the case of analyzing affected-unaffected sib pairs, the proposed approach can increase the statistical power to detect linkage by 50% compared to the method proposed by Spielman and Ewens (1998) for these discordant sibling pairs. Statistical properties of this unified likelihood-based approach will also be discussed. Supported in part by NIH grant GM59507.

Program Nr: 2584 from the 1999 ASHG Annual Meeting

Type 2 diabetes susceptibility loci maps on chromosomes 1 and 20 in Chinese Han families. *J.Y. Zhao[1,2], M.M. Xiong[2], W. Huang[3], H. Wang[4], J. Zuo[1], Z. Chen[3], B.Q. Qiang[5], M.L. Zhang[1], W.N. Du[1], J.L. Chen[3], W. Ding[3], W.T. Yuan[3], Y. Zhao[3], H.Y. Xu[3], L. Jin[2], Y.X. Li[4], Q. Sun[4], Q.Y. Liu[4], F.D. Fang[1]*. 1) Biochemistry and Molecular Biology, Institue of Basic Medical Science, Chinese Academy of Medical Science, Beijing, 100005, P. R. China; 2) Human Genetics Center, University of Texas-Houston, Houston, TX 77225; 3) Chinese National Genome Center at Shanghai, 201203, P. R. China; 4) Department of Endocrinology, Peking Union Medical Hospital, Beijing, 100730, China; 5) Chinese National Genome Center at Beijing, Beijing, 100005, China.

As a part of genome-wide scan for identifying genes that contribute to type 2 diabetes in samples of 32 Chinese Han families, we studied chromosomes 1 and 20. Thirty six markers at an average of 8cM on chromosome 1 and eight markers at an average of 12.5cM on chromosome 20 were genotyped. Linkage analysis was performed by use of two point and multipoint parametric and nonparametric methods in GENEHUNTER. Two point linkage analysis identified marker D1S193 on chromosome 1 and marker D20S118 on chromosome 20 showing significant evidence for linkage with LOD=6.1477 (P=5.2 X10-8) and NPL Z=4.1733 (P=1.5 X10-5), and LOD = 4.677 (P=1.73 X10-6) and NPL Z=2.8997 (P=0.00187), respectively. Two point lod score calculations also demonstrated suggestive linkage for the marker D20S115 with LOD=1.93 (P=0.0014).

Program Nr: 2585 from the 1999 ASHG Annual Meeting

Multipoint Linkage Analysis Using a Mapping Function, with an Application to Mapping IDDM. *L.P. Zhao, R.L. Prentice.* Division of Public Health Sciences, Fred Hutchinson Cancer Res Ctr, Seattle, WA.

The advances made in genomic technologies as well as in the construction of the human genome map will provide invaluable tools for discovering disease genes via genome scan studies with hundreds or even thousands of markers. A large array of markers is expected to be highly informative, but methods, handling multiple markers are relatively undeveloped to date. In this paper, we introduce a two-staged method for multipoint linkage analysis. At the first stage, the method estimates recombination fractions of putative disease genes with all individual marker loci using, for example, the semiparametric method recently proposed (Zhao et al. 1998a,b,c). Then, at the second stage, the method integrates estimated RFs based upon the genomic structure underlying all marker loci on a chromosome, using a mapping function to form a likelihood function. The likelihood can be used to construct test statistics for assessing the genome-wide significance, for estimating the number of disease genes, and for estimating locations of such genes along with confidence intervals for estimated locations. Under the assumed likelihood, the estimation procedure is expected to be efficient. Furthermore, it separates the calculation involving pedigrees from that dealing with multiple markers, and thus ensures practical computation for large pedigrees with a large array of markers. Scientifically, this method allows one to incorporate any partial knowledge about the disease phenotype under investigation. To illustrate this method, we apply this approach to a simulation experiment as well as to mapping study of insulin-dependent diabetes mellitus (IDDM) conducted in the United Kingdom. The IDDM data from the United Kingdom study and other studies will be more fully explored in a companion paper, and the result supports the presence of two major disease genes in chromosome 6q25-27. These positive findings, which remain to be proven ultimately via cloning, also support the utility of an affected sib pairs research strategy.

Program Nr: 2586 from the 1999 ASHG Annual Meeting

Genome-wide scanning for loci linked to essential hypertension in Chinese families. *D. Zhu[1], M. Xiong[2], S. Chu[1,2], L. Jin[2,3,4], G. Wang[3], W. Yuan[1], Y. Zhan[1], W. Zhang[1], S. Dong[1], P. Gao[1], G. Zhao[1], W. Huang[3]*. 1) Shanghai Institute of Hypertension, Ruijin Hospital, Shanghai Second Medical University, Shanghai, China; 2) Human Genetics Center, University of Texas-Houston, Houston, TX, USA; 3) National Human Genomic Center in Shanghai, Shanghai, China; 4) Institute of Genetics, Fudan University, Shanghai, China.

Essential hypertension is a complex, polygenic disease in which many genetic and environmental factors are involved and they may interact with each other in unpredictable ways in its development. To elucidate the genetic mechanisms of essential hypertension, 79 Chinese nuclear families residing in Shanghai were recruited in a genome-wide scanning study. 283 affected sib-pairs were counted in those families with 279 patients with essential hypertension. 393 family members were genotyped using ABI PRISM^{TM} Mapping Set Version 2 including 240 microsatellite markers with an average distance of 15 cM. Non-parametric linkage analyses (SIBPAIR and GENEHUNTER/NPL) gave statistically significant evidence for eight predisposing loci on Chromosome 2, 3, 7, 8 and 18. Among them, three markers (D2S168, D2S142 and D2S151) were on Chromosome 2. A replication study is needed to weed out the false positive linkage and to reduce the size of the candidate regions identified in this study.

Program Nr: 2587 from the 1999 ASHG Annual Meeting

Ventricular arrhythmia and dilated cardiomyopathy is linked to chromosome 2. *X. Zhu[1], D. Dreher[1], K. Hoang[2], J. Tan[2], S. Hunt[2], R.H Vagelos[2], M. Fowler[2], E.M. McNally[1].* 1) Medicine/Cardiology, University of Chicago, Chicago, IL; 2) Stanford University, Palo Alto, CA.

Eight different genetic loci (1p11, 1p32, 2q31, 3p22, 6q23, 9q13-22, 10q22, and 15q14) have been linked to the development of autosomal dominant familial dilated cardiomyopathy (FDC). Phenotypically, patients with FDC show enlargement of all four chambers of the heart and development of congestive heart failure with or without cardiac conduction system defects. However, the molecular etiology and the relationship between ventricular arrhythmias and the progression of dilated cardiomyopathy are not well understood. To this end, we have identified a large pedigree with ventricular arrhythmias and, in some instances, dilated cardiomyopathy whose phenotype segregates as an autosomal dominant disorder. The disorder is progressive and has an age of onset that ranges from the third to the sixth decade. The penetrance of this disorder for individuals in their sixth decade is estimated at 95%. A genome wide linkage scan was conducted using the Weber 8 markers on an ABI 377. Genotypes were scored blinded to phenotype and data was analyzed using FASTLINK 5.1. Approximately 50% of the genome was excluded before two point LOD score analyses identified markers on the long arm of chromosome 2 that showed maximal LOD scores of 3.50 at q =0, identifying ventricular arrhythmias with cardiomyopathy as a genetically distinct disorder. Haplotype analysis identified centromeric and telomeric recombination events. Thus, the interval containing the gene for ventricular arrhythmias and dilated cardiomyopathy was refined to 13 cM. Several interesting candidate genes including translin, brain-derived phosphotase 1, RAS-related protein RAB6, and a G-protein coupled receptor gene are currently being evaluated. Haplotype analysis also excluded nebulin, an important muscle-expressed gene in this region. This study demonstrates a new genetic locus associated with ventricular arrhythmia and dilated cardiomyopathy.

Program Nr: 2588 from the 1999 ASHG Annual Meeting

The Interleukin-4 receptor variants I50V and Q576R in atopic children. *S. ZIANI[1], N. Chavernoz[1], G. Morgant[1], A. Grimfeld[2], P. Aymard[1], D. Feldmann[1].* 1) Dpt of Biochemistry, Hopital A Trousseau, Paris, France; 2) Dpt of Allergology, Hopital A Trousseau, Paris, France.

Atopy is a very common disease with genetic predisposition. Interleukin-4 (IL4) and IL4 receptor (IL4R) play a central role in the regulation of IgE synthesis. Recently, two polymorphisms in the gene coding for IL4R alpha chain have been identified : I50V and Q576R or Q551R. The gain of function of IL4R alpha chain baring a residue Ile50 or Arg576 and the association of I50V or Q576R variants with atopy or asthma is still controversial. So, We have studied the two variants in a population of atopic children. We have studied 93 atopic patients (1-16y) with elevated serum IgE and a positive screening test for pulmonary allergy (Phadiatop, Pharmacia) and/or a positive radioimmunoadsorbent test (score>3) in respons to one or more allergens, and 92 non atopic controls (1-20y) with normal serum IgE and negative screening test for pulmonary allergy. I50V and Q576R polymorphisms were studied by PCR mediated site directed mutagenesis. Frequency of I50 allele was not different in atopic (40%) and control (43%) children. Frequency of homozygotes I50 was 31.6% in atopic patients and 30% in controls. Q576R variant was detected in 49 atopic patients (R576 allele frequency : 36%) and in 41 controls (R576 allele frequency : 24%). Frequency of homozygotes R576 was 20% in atopic and 3% in controls ($p<0.001$ $chi2=13.5$ $DL=2$). In conclusion, an increase of homozygotes R576 was observed in our atopic population versus non atopic subjects. This result suggest that R756 variant of IL4R gene could play a role in the genetic predisposition of atopy.

Program Nr: 2589 from the 1999 ASHG Annual Meeting

Fine-mapping of the cystic fibrosis modifier locus on human chromosome 19. *J.S. Zielenski, D. Markiewicz, M. Corey, I. Aznarez, J. Shin, R. Rozmahel, P. Durie, L.C. Tsui, and. the CF Modifier Collaborative Group.* The Hospital for Sick Children, Toronto, Ontario, Canada.

Clinical phenotype in CF is influenced by mutations at the *CFTR* locus as well as secondary genetic modifiers. We have recently obtained strong evidence indicating the presence of a CF modifier locus (*CFM1*) on human chromosome 19, region q13.2. The linkage was demonstrated by identity by descent analysis with polymorphic markers. *CFM1* appeared to contribute to the predisposition of meconium ileus (MI) which is a severe form of intestinal obstruction in the perinatal period. The strongest linkage was detected for markers in a 3.75 Mb interval between markers *D19S408* and *D19S412*. To narrow the critical region for *CFM1*, we have performed transmission disequilibrium test (TDT) for markers in the above interval. The study population included 33 families with at least one CF child with MI from the original linkage study. In addition, we recruited 124 "trios", i.e., CF patients with MI and their parents, for the analysis. To date, we tested 17 markers: *D19S408-40867/DraI -APOC2-APOC1/HpaI-2050/AvaII-31237(GT)n-10080(GT)n-D19S219-DM(GCT)n-D19S112-CALM3(178+66C/T)- D19S412-STD(-910C/A)-D19S902-D19S604-D19S246-KLK1*. Two computer programs, GASSOC and GENEHUNTER, were used to assess a linkage disequilibrium. Studies were performed with 142 families for 3 microsatellite markers, *31237(GT)n*, *10080(GT)n*, and *DM(GCT)n*, spanning a 500-kb region within the interval of strong linkage. Intragenic SNPs for *CALM3(178+66C/T)* and *STD(-910C/A)* were also studied in 93 families. No transmission disequilibrium could be detected for any of these markers by either computer programs. The most significant association was detected for *APOC2* (GASSOC *P*=0.002), with GENEHUNTER pointing to two specific alleles (*P*=0.01), but the number of families tested was small (n=33). Additional studies are in progress, particularly with markers surrounding the *APOC2* locus. The CF Modifier Collaborative Group: T. Casals, D. Bozon, A. Krebsowa, M. Claustres, B. Marshall, E. Bjorck, B. Mercier, A. Palacio, E. Langfelder, J. Bal, G. Cutting, S. Larriba, L. Chertkoff, C. Ferec, B. Strandvik, M. Macek Jr., X. Estivill.

Program Nr: 2590 from the 1999 ASHG Annual Meeting

Missense mutations in the cystic fibrosis gene in adult patients with asthma. *C. Lazaro[1], R. de Cid[1], J. Sunyer[2], J. Soriano[2], J. Gimenez[1], M. Alvarez[1], T. Casals[1], J.M. Anto[2], X. Estivill[1].* 1) Medical and Molecular Genetics Center-IRO, Barcelona, Spain; 2) Respiratory and Environmental Health Research Unit-IMIM, Barcelona, Spain.

Asthma is a multifactorial disorder with a complex phenotype that affects 5% of adults and 10% of children worldwide. As a complex genetic disorder, it is hypothesized that genetic and environmental factors play complementary roles. There is evidence to suggest a link between asthma and cystic fibrosis (CF) although first attempts to assess an association between asthma and the CFTR gene led to inconsistent results. Thus, the characterization of the CFTR coding region in a well defined asthma population has so far not been reported. Here we present the complete analysis of the CFTR gene, by using multiplex denaturing gradient gel electrophoresis (DGGE) and single strand conformation analysis (SSCA) in a sample of adult patients with asthma and in subjects from the general population. This characterization identified missense mutations in 15% of 144 unrelated adult patients with asthma, but in none of 41 subjects from the general population (P = 0.005). Some of these changes (R75Q, G576A, R668C and L997F) have also been found in an extended sample of individuals from the general population, in similar proportions. There were no major differences in the asthma-related phenotypes according to the presence of missense mutations. The distribution of genotypes for the 470M/V variant was significantly different between the patients with asthma bearing CFTR mutations and those without, the M470 variant being detected in 90% of the patients with asthma and amino acid variants, but in 63% of those without amino acid changes (P=0.01) and in 63% of subjects from the general population (P=0.02). None of the patients with missense mutations had the 5T allele of intron 8 of CFTR, responsible for low CFTR levels, while it was detected in 8% of asthma patients without CFTR mutations and in 9% of subjects from the general population. These findings suggest a putative role of the CFTR gene in the genetic variability of asthma.

Program Nr: 2591 from the 1999 ASHG Annual Meeting

Endogenous huntingtin modulates the cellular toxicity of mutant huntingtin. *B.R. Leavitt[1], A. Hackam[1], C.A. Gutekunst[2], K. McCutcheon[1], A. Yassa[1], R. Singaraja[1], G. Kimel[3], S. Hersch[2], W. Vogl[3], M.R. Hayden[1].* 1) CMMT, UBC, Vancouver, BC, Canada; 2) Emory U. School of Medicine, Atlanta, GA, USA; 3) Dept. of Anatomy, UBC, Vancouver, BC, Canada.

We have developed yeast artificial chromosome (YAC) transgenic mice expressing normal (YAC18 CAG) and mutant (YAC46 and YAC72 CAG) human huntingtin (htt) in a developmental and tissue specific manner identical to endogenous htt. YAC72 mice develop a behavioral phenotype and selective degeneration of medium spiny neurons in the striatum similar to that observed in HD. YAC transgenic mice expressing human htt can compensate for the loss of endogenous htt, and rescue the embryonic lethality of htt knockout mice. Decreasing the levels of endogenous htt in YAC46 and YAC72 mice, but not YAC18 mice, leads to a novel phenotype characterized by testicular atrophy, inability of male mice to breed, and massive cell loss in the testes. Ultrastructural analysis (EM) of the testes of YAC72 mice lacking endogenous htt revealed massive spermatocyte loss, phagocytosis of dying cells, and intracellular inclusions not seen in YAC72 mice with normal endogenous huntingtin levels, or in YAC18 mice lacking endogenous htt. The cell death in the testes is due to increased apoptosis (TUNEL staining) and is modulated by level of endogenous htt. Testicular cell death is also CAG repeat size dependent with a greater effect in YAC72 vs. YAC46 and no effect in YAC18 mice. Co-expression of mutant and normal htt in a neuronal cell culture model leads to decreased neuronal death, less huntingtin aggregation, and less htt cleavage than seen with co-expression of mutant htt and vector alone. Protection from neuronal death is seen with C-terminal htt fragments but not N-terminal fragments of htt, suggesting that the C-terminal region of htt is responsible for this effect. These data provide evidence that endogenous htt modulates the cellular toxicity of mutant htt, and suggest that normal htt may be an anti-apoptotic protein with therapeutic potential.

Program Nr: 2592 from the 1999 ASHG Annual Meeting

Spinocerebellar ataxia 7 (SCA7) : cellular localization and search for partners of ataxin-7. *A.S. Lebre[1], G. Cancel[1], C. Zander[1], G. Yvert[2], C. Duyckaerts[3], P. Kussel[4], J.H. Camonis[5], A. Brice[1].* 1) INSERM U289, Hopital de la Salpetriere, Paris, cedex 13, France; 2) IGBMC, Illkirch, CU de Strasbourg, France; 3) Laboratoire de Neuropathologie, Hopital de la Salpetriere, Paris, France; 4) Institut Pasteur, Paris, France; 5) Institut Curie, Paris, France.

Spinocerebellar ataxia 7 is a progressive neurodegenerative disease caused by an expansion of a CAG repeat in the coding region of the gene. The distinct neuropathological feature of SCA7 is degeneration of optic pathways and the retina. Ataxin-7 is a protein of unknown function which contains a bipartite nuclear localization signal, four polyproline domains (SH3-binding domains), and a serine-rich C-terminus. It is expressed in various tissues including CNS (David et al., 1997). The mutated protein has been reported to be nuclear, as analyzed by Western blot on cell fractions of lymphoblasts from SCA7 patients. We have carried out immunocytochemical studies in transfected Cos7 cells, and showed a strong/almost exclusive nuclear localization of both wild type and mutated ataxin-7. In contrast, expression of ataxin-7 is predominantly cytoplasmic in normal human brain. Confocal microscopy studies are underway to localize ataxin-7 more precisely. In contrast to its ubiquitous expression, SCA7 mutations lead to a selective neuronal death. This phenotype could be explained by an interaction with a specific partner. Using a two-hybrid approach, we screened a human neural retina cDNA library for ataxin-7 binding proteins. Screening with the mutated ataxin-7 (100 Glutamines) yielded 21 different clones that interact with normal and mutated ataxin-7. BLAST analysis identified 17 known proteins, 2 EST and 2 unknown proteins. One partner interacts more strongly with mutated than normal ataxin-7. This latter protein and three others have been selected for further studies on the basis of their expression in the CNS as assessed by Northern blot. GST pull down studies are underway to confirm the (direct) interaction between ataxin-7 and these partners. Immunoprecipitation and immunochemistry studies will be performed to determine whether these proteins do meet in vivo, and in which (sub)cellular compartment.

Program Nr: 2593 from the 1999 ASHG Annual Meeting

Construction of an imprinting status transcript map of 15q11-q13. *S. Lee, R. Wevrick.* Medical Genetics, University of Alberta, Edmonton, Alberta, Canada.

Human chromosome bands 15q11-q13 are prone to interstitial deletions. This region contains a group of imprinted, uniparentally expressed genes. Genomic imprinting is the epigenetic modification of DNA that results in differential expression of the paternal and maternal alleles of a gene. Deletions of either the paternal or maternal copy of 15q11-q13 are the predominant cause of two imprinted disorders, Prader-Willi Syndrome (PWS) and Angelman Syndrome (AS). PWS is a neurobehavioural disorder characterized by neonatal hypotonia, developmental delay, and hyperphagia. PWS is due to the loss of expression of one or more paternally expressed genes, most commonly as a result of deletions on the paternal copy of chromosome 15. AS is characterized by developmental delay and ataxia. AS is caused by the absence of expression of a single, maternally expressed gene, *UBE3A*, usually as a result of deletions on the maternal copy of the chromosome. Studies suggest that imprinted genes may be clustered into domains, suggesting a regional method of gene regulation. Currently, 5 imprinted genes, *ZNF127*, *NDN*, *SNRPN*, *IPW* and *UBE3A*, and 4 nonimprinted genes have been identified within this 4 Mb deletion region. We hypothesize that there are additional genes in the 15q11-q13 region and that some of these genes are imprinted. We have physically mapped at least 5 Expressed Sequence Tags (ESTs) from the GeneMap 98 database which do not correspond to known genes to a YAC contig spanning the 15q11-q13 region. We have analyzed the GeneMap 98 ESTs and additional ESTs mapped to the region by other groups for uniparental expression by RT-PCR. Lymphoblast and brain RNA were collected from normal, PWS and AS patients. Fibroblast RNA from normal and PWS were also used in the analysis. Using this method, we have established that a subset of the ESTs examined are paternally expressed and may correspond to genes that are involved in PWS. Results from this analysis will give an overview of the imprinting status of a large number of ESTs 15q11-q13, and may provide insights into the organization and control of this imprinting domain.

Program Nr: 2594 from the 1999 ASHG Annual Meeting

Analysis of Familial Combined Hyperlipidemia with chromosome 1q21-q23 markers and the APOAI/CIII/AIV gene cluster in the NHLBI Family Heart Study. *M.F. Leppert[1], H. Coon[1], I.B. Borecki[2], D.K. Arnett[3], S.C. Hunt[1], M.A. Province[2], L. Djoussé[4], R.H. Myers[4]*. 1) Depts of Human Genetics, Psychiatry and Cardiovascular Genetics, Univ Utah, Salt Lake City, UT; 2) Division of Biostatistics, Washington Univ, St. Louis, MO; 3) Division of Epidemiology, Univ of MN, Minneapolis, MN; 4) Section of Preventive Medicine and Epidemiology, Boston Univ, Framingham, MA.

FCHL is the most common familial dyslipidemia with a prevalence of 1%-2%, and is implicated in up to 20% of cases of premature coronary heart disease. Although underlying mutations for FCHL have yet to be identified, Pajukanta et al. (1998) recently published positive linkage to chromosome 1q markers, with the highest lod score of 5.93 occurring between D1S104 and D1S1677. Using the same diagnostic criteria, the Family Heart Study has defined 71 FCHL families comprising 170 cases for a total of 137 possible affected sibling pairs. The FCHL criteria require elevation in both serum LDL cholesterol and triglycerides within the family, with at lease two affected first degree relatives. Markers D1S104 and D1S1677 were typed as a replication attempt. Suggestive allele sharing was found in FCHL sibships with these markers (multipoint NPL score = 2.26, p = 0.01). In addition, previously reported linkage of FCHL to apolipoprotein AI/CIII/AIV (Wojciechowski et al., 1991) has been investigated in FHS families. Results revealed positive but nonsignificant allele sharing among FCHL sibships with AI/CIII/AIV using marker D11S4127 (p = 0.19).

Program Nr: 2595 from the 1999 ASHG Annual Meeting

Transmission of skewed X-inactivation in familial Rett Syndrome. *N. Levy[1,2], L. Villard[1], F. Xiang[3], V. Labelle[2], M. Tardieu[4], J. Chelly[5], M. Fontés[1]*. 1) Inserm U491, "Génétique médicale et développement, Faculté de médecine de la Timone, Marseille, France; 2) Département de génétique médicale, Hôpital d'enfants de la Timone, Marseille, France; 3) Department of molecular medicine, Karolinska hospital, Stockholm, Sweden; 4) CRI9612, "Virus, neurone et immunité", UFR Kremlin Bicêtre, Paris, France; 5) InsermU129,"physiologie et pathologie génétiques et moléculaires, CHU Cochin-Port-Royal, Paris, France.

Rett syndrome (MIM312750) is a disease of unknown origin which essentially affects girls. Based on clinical features, this disorder seems to have a neurodevelopmental origin, since affected patients present an arrest in brain's development. More than 99% of affected patients correspond to sporadic cases with no other relatives affected even in large families. In most of the rare families reported so far, the disease appears as a classical Rett phenotype in girls from the same sibship. A genetic linkage to Xq28 has been proposed (Xiang et al., 1998; Siriani et al., 1998; Webb et al., 1998). In a recent report, Siriani et al. (1998) suggested that a skewed X inactivation could be a necessary event in the transmission of Rett syndrome from carrier mothers to affected girls. Indeed, they observed in one family a carrier mother having a preferential inactivation of the morbid allele, even though her daughters had a random inactivation pattern. In order to test the liability of this hypothesis, we explored more families and analysed the X inactivation status in the mothers and daughters. Surprisingly, an important bias of inactivation was observed both in mothers and affected daughters. These observations clearly raises the question of the relationships between X inactivation bias and the Rett mutation in familial cases, as well as the localization of the RETT mutation. Hypothesis will be presented, which could reconciliate the transmission of the disease in familial forms with the sporadic cases occurrence. In these hypothesis, the role of a second event, the skewed X inactivation is taking a major role.

Program Nr: 2596 from the 1999 ASHG Annual Meeting

Investigation of the *TSC2* locus in lymphangioleiomyomatois (LAM) patients. *H. Li[1], H. Basterrechea[2], M. Glassberg[1], L. Baumbach[2]*. 1) Medicine, Univ of Miami School of Med, Miami, FL; 2) Pediatrics, Univ. of Miami Scool of Med, Miami, FL.

Pulmonary lymphangioleiomyomatois (LAM) is a fatal lung disease that predominantly affects women in their child-bearing years. The disease is characterized by the focal accumulation of airway mesenchymal cells ("LAM cells") and dense collagen deposition associated with air-filled cysts within affected lung tissue. Little is known about its pathogenesis, but the symptoms, radiographic findings, and histopathologic descriptions of LAM are identical to those of a subset (1-5%) of female tuberous sclerosis patients (TSC). The only report to address a possible genetic relationship between TSC and LAM detected somatic loss of heterozygosity at *TSC2* in 7/13 renal angiomyolipomas from LAM patients.

Our studies are focused on the possible identification of germline DNA sequence variations in *TSC2* in genomic DNA from LAM patients with and without features of TSC We first analyzed the *TSC2* GAP-binding region (exons 34-41) in genomic DNA samples isolated from peripheral blood samples from 20 LAM patients (14 with additional symptoms resembling TSC) and 7 unrelated age-matched controls, through the development of an exon-specific PCR amplification assay. No gross exon alterations were detected. These initial studies were followed by SSCP analysis of each exon, scanning for possible genetic variations in the GAP-binding region. SSCP variants were further analyzed by DNA sequencing, and DNA sequences compared to the published wild-type *TSC2* sequence. Although several SSCP variants were detected in 2 LAM patients in this region, DNA sequencing of SSCP variant bands and genomic DNA-PCR products detected only wild-type DNA sequence in these patients. These results suggest that germline mutations in, at least, the *TSC2* GAP-binding domain, are not a contributory factor to the association between TSC and LAM.

Program Nr: 2597 from the 1999 ASHG Annual Meeting

Imprinting study of Prader-Willi syndrome with methylation-specific PCR(MSPCR). *L.Y Li, M.H Song, J.J Fu, X.R Li, G.X Lu.* Human reproductive engineering laboratory, Hunan Medical University, Changsha, Hunan, P.R.China.

In this paper reported here that we used a faster,more efficient method to diagnose Prader-Willi syndrome(PWS). Differential DNA methylation at several sites in the PWS critical region exists inwhich the maternalhomologue is methylated and inactive while the paternal homologue is unmethylated and transcriptionally active.MSPCR is based on sodium bisulfite treatment of DNA, which converts unmethylated, but not methylatedcytosine residues to uracil, and PCR primers specific for the maternal and the paternal allele. Methylation analysis by Southernblot isusedto validate the MSPCR result. The results indicated Bisulfite-modified DNA from PWSpatients amplified only with methylated allele-specific primer pair whichshowed only maternal 174-bp PCR product. MSPCR and PW71B methylation studies showed an abnormal pattern consistent with the clinic diagnosis of PWS. Untreated DNA does not produce a PCR product. Conclusion:It is proved that MSPCR be able to detect all presently testable causes of PWS(deletion, uniparental disomy (UPD), or imprinting mutation) in an efficient first step for stepwise dignostic testing, comparing to either Southernblot analysis which is more timeconsuming, or fluorescence in situ hybridization(FISH) which can not detect UPD or imprinting mutation. [Key words] Methylation-specific PCR Methylation Prader-willi syndrome.

Program Nr: 2598 from the 1999 ASHG Annual Meeting

Novel mtDNA mutations detected by TTGE. *M.-H. Liang, J.J. Wang, T.J. Chen, W. Fan, L.-J. Wong.* Georgetown University Medical Center, Institute for Molecular and Human Genetics, Washington, DC.

Most reported pathogenic mtDNA mutations are heteroplasmic. Our recent studies using TTGE to scan mutation in 3 regions containing $tRNA^{Leu}$(L), $tRNA^{Lys}$(K), and penta-$tRNA^{Trp, Ala, Asn, Cys, Tyr}$(WANCY) regions suggested that heteroplasmic mtDNA may be more common than previously reported(Chen et al., 1999). In this study, two hundred and eighteen specimens suspected of mitochondrial disorders are screened for mtDNA mutations in ten regions containing 21 tRNAs and part of the mRNAs, totaling 37% of the entire mitochondrial genome by temporal temperature gradient gel electrophoresis (TTGE). These specimens were negative for 11 common point mutations (MELAS,MERRF, NARP, LHON, cardiomyopathy) and large deletions (KSS, Pearson). Our results demonstrate that 6.9% (15/218) of the specimens showed multiple bands on TTGE analysis suggesting heteroplasmic base substitutions. The heteroplasmic mutations occur most frequently (1.9%) in the WANCY region. They were not detected in L and G/ND3/R ($tRNA^{Gly, Arg}$) regions. Sequencing results confirmed several mutations responsible for the molecular state of heteroplasmy. Novel mutations identified include T5580C ($tRNA^{Trp}$+1), G5821A ($tRNA^{Cys}$), C5840T($tRNA^{Tyr}$), C7476T($tRNA^{Ser}$), A12333G ($tRNA^{Leu(CUN)}$), and T4454C($tRNA^{Met}$). Two hundred and fifty three homoplasmic band shifts have been detected in 8 different regions. In conclusion, TTGE has been effectively used to scan mtDNA. It will be suitable for the mutational analysis of the entire mitochondrial genome.

Program Nr: 2599 from the 1999 ASHG Annual Meeting

A candidate gene study in Finnish families with premature coronary heart disease and low HDL-cholesterol. *H.E Lilja[1,2], P. Pajukanta[2], A. Soro[3], L. Peltonen[1,2], M.-R. Taskinen[3]*. 1) Human Molecular Genetics, Nat. Public Health Institute, Helsinki, Finland; 2) Department of Human Genetics, University of California, Los Angeles; 3) Department of Medicine, University of Helsinki, Finland.

Low serum high density lipoprotein cholesterol (HDL-C) is one of the most important risk factors for coronary heart disease (CHD). The prevalence of familial low HDL disorder is approximately 4% in patients with premature CHD and at least 50% of the variation in HDL-C levels is estimated to be genetically determined. However, the genetic component of low HDL is still largely unknown. Most likely low HDL-C disorder is a complex trait, in which multiple genes and environmental factors are playing a role. To explore the genetic backround of low HDL-C in the genetically isolated Finnish population, we have so far collected 14 well-documented Finnish pedigrees with premature CHD and isolated low HDL-C. Inclusion criteria for the proband are the following: age of 30-60 years, angiographically or clinically verified CHD and HDL-C level below to that of the age- and sex-specific 10th percentile. To focus on isolated low HDL families, strict limits for serum total cholesterol and triglycerides are used. We selected a candidate gene approach and analyzed genes encoding proteins involved in metabolic pathways of HDL-C, such as apolipoproteins and lipolytic enzymes, using intragenic and flanking microsatellite markers. We genotyped 94 individuals, of whom 59 are affected fulfilling the strict criteria. Both parametric linkage and nonparametric affected sibpair (ASP) analyses were performed to avoid disadvantages of each of the methods alone. Because we used a candidate gene approach, we accepted $p<.05$ to test for significance. Our preliminare data indicate that there is suggestive evidence of linkage emerged with the markers on the apolipoprotein A2 and lipoprotein lipase genes and the low HDL-C trait in ASP analyses, with p-values of 0,019 and 0,045. We are currently performing a genome scan to define the genome wide significance of these findings and to identify other genetic loci.

Program Nr: 2600 from the 1999 ASHG Annual Meeting

The human *SRA1* gene maps proximal of the Prader-Willi/Angelman syndrome domain in 15q11 and is non-imprinted. *D.P. Locke[1], T. Ohta[1], J.H.M. Knoll[2], R.D. Nicholls[1]*. 1) Case Western Reserve University, Cleveland, OH; 2) Children's Mercy Hospital, Kansas City, MO.

Sra1, the product of the *SRA1* (KIAA0068) gene, is found in association with Rac1, a Rho GTPase family member (JBC 273:291, 1998). Sra1 interacts with Rac1 and F-actin and is implicated in membrane ruffling. The murine homolog, *Sra1* (formerly *Shyc*), is activated during neuroectodermal differentiation of P19 cells (Neuro Lett 252:69, 1998). Phylogenetic comparisons indicate high conservation between the human, murine and putative *C. elegans* homologs. Data from the G3/GB4 radiation hybrid panel (www.ncbi.nlm.nih.gov/genemap98/) suggested *SRA1* mapped to 15q11-q12 close to, or within, the Prader-Willi/Angelman syndrome (PWS/AS) imprinted domain. We confirmed this location by somatic cell hybrid mapping the *SRA1* 3'-UTR, and by FISH localization of *SRA1* with a BAC probe. FISH also demonstrated that *SRA1* is likely a single copy gene. Further mapping of the 3'-UTR to YACs 931C4 and 124A3 places *SRA1* proximal of the *HERC2*-duplicons associated with PWS/AS deletion breakpoint 2 (Amos-Landgraf, AJHG in press), and hence proximal of the known PWS/AS imprinted domain. We found that *SRA1* is biallelically expressed using RT-PCR with somatic cell hybrid RNA (PNAS 95:14857, 1998) as well as RNA isolated from PWS/AS imprinting mutation patients. A murine BAC containing the *Sra1* 3'-UTR was isolated and mapped by FISH to the syntenic chromosome 7C location, within a deletion of the PWS/AS-homologous domain (Gabriel, PNAS in press). Murine imprinting assays indicate *Sra1* is non-imprinted. We are currently assessing the location of *SRA1* in Class I and II PWS/AS deletions, and whether *SRA1* shows asynchronous replication timing as for the PWS/AS domain. We conclude that *SRA1* is a single copy gene located outside the PWS/AS imprinted domain with biallelic expression in human and mouse. The location of this nonimprinted gene defines one boundary of the PWS/AS imprinted domain. Further study of the interval between *ZNF127*, the closest imprinted gene, and *SRA1* in human and mouse will allow identification of epigenetic control elements that regulate the boundary of this imprinted domain.

Program Nr: 2601 from the 1999 ASHG Annual Meeting

Examination of Collagen Genes in Kindred with Developmental Dislocation of the Hip. *A.L Lonkar[1], K.E Murphy[2]*. 1) Microbiology & Molecular Cell Sciences, University of Memphis, Memphis, TN; 2) Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, TX.

Work from several laboratories has shown that mutations in various genes encoding collagen proteins cause hip joint hypermobility, which is a characteristic of several developmental diseases. Because hip joint malformation is the primary feature of developmental dislocation of the hip (DDH), we are investigating the possibility that mutations within those genes are contributing factors to this heritable disease. Interestingly, an appropriate canine model, canine hip dysplasia (CHD), is available. Published findings have indicated an abnormal collagen III:I ratio in dogs with CHD and a similar abnormal ratio has been reported in DDH patients. These data suggest DDH may result, in part, from aberrant metabolism of collagens I and III. Furthermore, considerable data demonstrate that certain regions spanning exons and introns within the human *col*1A2 gene harbor mutations leading to abnormal hip joint development. To date, we have identified by PCR and direct sequencing an extensive polymorphism within one of our subject families. Analyses of this polymorphism within subject A-1 has shown multiple point mutations within exons 9, 10 and 11 of the *col*1A2 gene not present within any other subjects (families A, B, C or controls). It has been determined that there is an amino acid switch from P to A in exon 9 caused by this polymorphism but the implications of this amino acid alteration have yet to be determined.

Program Nr: 2602 from the 1999 ASHG Annual Meeting

Toll-4 mutations D299G and P714H give reduced levels of NFkB activation in transfected CHO/CD14 cells. *E. Lorenz, K.L. Schneider, N.C. Arbour, D.A. Schwartz.* Dept Int Med, Univ Iowa, Iowa City, IA.

We identified a common (allelic frequency 5-10%) mutation in the extracellular domain of toll-4 (D299G) in humans. Similar to the missense P712H mutation in murine toll-4 seen in strain C3H/HeJ mice (amino acid residue 714 in humans), the D299G mutation in humans is associated with an LPS hyporesponsive phenotype. We used site-directed mutagenesis to recreate both mutations in plasmids for use in in vitro transfection studies in CHO/CD14 cells. Amino acid 299 is part of the extracellular domain, while amino acid 714 is part of the intracellular domain of toll-4. Both residues 299 and 714 in humans are highly conserved between mice and humans. Using NFkB as a reporter gene, the wild type gave a 2-fold activation in response to 100 ng/ml of LPS for 5 hours. The mutation corresponding to the one identified in mouse strain C3H/HeJ gave no activation in response to the same dose of LPS, while the D299G mutation gave intermediate levels of LPS response. This is the first in vitro proof that the mutation in mouse strain C3H/HeJ abolishes the response to LPS. In addition, this result proves that mutations in both the extracellular domain, as well as the intracellular domain, of toll-4 affect LPS responsiveness in vitro.

Program Nr: 2603 from the 1999 ASHG Annual Meeting

Examination of 15q11-13 DNA methylation and expression imprints in prolonged lymphoblastoid cell culture.
A.C. Lossie, K. Dopp, M.M. Whitney, D.J. Driscoll. RC Philips Unit, Pediatric Genetics, University of Florida, Gainesville, FL.

One of the best studied imprinted regions in humans is the Angelman (AS)/Prader-Willi (PWS) domain located within 15q11-13. AS and PWS are distinct neurobehavioral disorders arising from the lack of a maternal (AS) or paternal (PWS) genetic contribution from 15q11-13. The imprinting center (IC), which overlaps exon 1 of *SNRPN* establishes methylation and expression imprints along the 2Mb AS/PWS domain. Several loci within 15q11-13 have DNA methylation imprints in peripheral blood leukocyte (PBL) DNA. Methylation analyses of 5' *SNRPN*, PW71, *NECDIN* and *ZNF127* indicate that the maternal alleles are preferentially methylated, while the paternal copies remain largely unmethylated in PBL DNA. In correlation with DNA methylation, these genes are preferentially expressed from the paternal chromosome.

Lymphoblastoid cell lines (LB) from AS and PWS patients are commonly used for genetic analysis. However, evidence for the stability of DNA methylation in LB cultures is questionable. We hypothesized that: 1) DNA methylation imprints in B-cell derived LB cell lines would be faithfully maintained within the IC, but would relax with increasing distance from the IC. 2) Changes in DNA methylation would correlate with expression.

We established LB cell lines from AS, PWS and control samples. During 12 months of continuous culture, samples were collected every 4 months; DNA and RNA were isolated at each time point. At the 4, 8 and 12 month time points, changes in methylation occurred at 5' *SNRPN*, PW71 and *ZNF127*, but not at *NECDIN*. However, RT-PCR showed that the demethylation at 5' *SNRPN* did not correlate with expression. Surprisingly, DNA methylation imprints in LB cultures are not faithfully maintained at regions within the IC, and methylation at the 3' end of the CpG island of 5' *SNRPN* is not crucial for maintenance of imprinted expression of *SNRPN*. Expression analyses of *ZNF127* and *NECDIN* are ongoing and should provide further insight into the role that DNA methylation plays in the transcriptional regulation of the AS/PWS imprinted domain.

Program Nr: 2604 from the 1999 ASHG Annual Meeting

Allelic variation of the fibulin-1 gene in age related macular degeneration. *A.J. Lotery, K. Vandenburgh, H. Haines, L. Streb, R. Hockey, G. Beck, V. Sheffield, E.M. Stone.* Department of Ophthalmology, University of Iowa, Iowa City, IA 52242.

Introduction: We recently demonstrated that the EFEMP1 gene is mutated in Malattia Leventinese and Doyne Honeycomb Retinal Dystrophy. These diseases are familial drusen syndromes and are phenotypically similar to the more common drusen disorder known as age related macular degeneration (AMD). EFEMP1 was not found to be associated with AMD. However EFEMP1 is homologous at the protein level to the fibulin gene family and indeed has previously been known as fibulin-3. Because of this homology we screened fibulin-1 for mutations in patients with AMD. Methods: The genomic structure of fibulin-1 exons 2 to 20 were ascertained from genomic databases and suitable SSCP primers were synthesized. 190 control patients and 494 patients affected with AMD from the University of Iowa retinal clinic were screened for sequence variation. The coding region of exons 2 to 20 were screened by single strand conformation polymorphism analysis (SSCP). Amplimers showing a band-shift were reamplified and sequenced bidirectionally using an ABI 377 automated sequencer and dye-terminator chemistry. Results: 11/190 control patients (5.7%) and 28/494 AMD patients (5.7%) were found to have missense changes in the fibulin-1 gene. In 8 cases these sequence variations were non-conservative. Conclusions: Fibulin-1 sequence variation was not significantly associated with the AMD phenotype in this study.

Program Nr: 2605 from the 1999 ASHG Annual Meeting

Neural Tube Defects and the 13q Deletion Syndrome: Delineation of a Critical Region for Neural Tube Defects in 13q33-34. *J. Luo[1,2], J. Charrow[3], J.F. Sarwark[2], J.S. Nye[1,3].* 1) Dept of Molecular Pharmacology, Northwestern University; 2) Dept of Orthopaedics, Northwestern University and Children's Memorial Hospital; 3) Dept of Pediatrics, Children's Memorial Hospital, Chicago, IL.

Neural tube defects (NTDs) are a variable feature in the 13q- syndrome. However, the relationship between the 13q- syndrome and NTDs is poorly understood. We present a case with both a characteristic 13q deletion phenotype and lumbosacral myelomeningocoele. The proband was a boy with severe mental retardation, microcephaly, hypertelorism, prominent cheek bones and incisors, short neck, hypoplastic fifth digits, club feet, and ambiguous genitalia. Cytogenetic analysis revealed 46,XY,del(13)(q33.2®qter). FISH studies with a total chromosome 13 probe showed hybridization to both chromosome 13s with no visible translocation. Parental chromosomes were normal. Using microsatellite markers, the deletion breakpoint was shown to be of paternal origin and was mapped to a 350kb region between D13S274 and D13S1311. Multiple proximal microsatellites were heterozygous and distal microsatellites were hemizygous.

This case is distinct from reported cases because the patient has multiple major malformations consistent with the 13q deletion phenotype, but the deletion is distal to and non-overlapping with the previously defined critical region in 13q32 (Brown et al., AJHG 57:859-866, 1995). In addition, an extensive literature search has suggested that many cases of 13q- and NTD have in common a deletion distal to 13q32. These results suggest that one or more genes in 13q33-13q34 are responsible for NTDs and some of the characteristic phenotypes of 13q- syndrome when hemizygous. Further work will elucidate the relationship of distal 13q deletions and neural tube defects. Supported by NINDS
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Program Nr: 2606 from the 1999 ASHG Annual Meeting

Generation and analysis of a BAC/PAC contig for the chromosome 15q11-q13 Autistic Disorder candidate gene region. *L.O. Maddox, M.M. Menold, L. Zaeem, M.P. Bass, M.A. Pericak-Vance, J.M. Vance, J.R. Gilbert.* Duke University Medical Center, Durham, NC.

Autistic Disorder (AD) is a complex neurodevelopmental disorder that affects approximately 2-10/10,000 individuals. Epidemiological studies have consistently implicated genetic factors in the etiology of AD, and it is estimated that as many as 2-10 genes may underlay this etiology. Evidence for an AD genetic risk factor on chromosome 15 is based on the following: 1) associated cytogenetic abnormalities; 2) increased recombination frequency in this region in AD versus non-AD families; 3) suggestive linkage results with markers D15S156, D15S219, and GABRB3; and 4) significant linkage/association with polymorphisms in the GABRB3 gene.

To isolate the 15q11-q13 candidate AD gene, a genomic contig and physical map of the 1.2 Mb region from the g-aminobutyric acid (GABA) gene cluster to the OCA2 locus was generated (cen-GABRB3-GABRA5-GABRG3-APBA2-OCA2-tel). Twenty-one BAC clones, 32 PAC clones, and 2 P1 clones have been isolated using markers D15S540, GABRB3, GABRA5, GABRG3, D15S822, D15S975, D15S156, D15S219, D15S217, and 32 novel markers developed from the end sequences of BAC/PAC clones. Analysis of critical markers in the contig utilizing the GeneBridge 4 radiation hybrid panel verified the chromosome 15 localization of clones creating a minimum tiling path of the candidate gene region. Six clones create a minimum tiling path from GABRB3 to GABRA5. The contig from GABRA5 exon 7 extending to exon 24 of the OCA 2 gene has a minimum tiling path of 11 clones. The region is rich in repetitive DNA elements including retrotransposons, LINEs, SINEs, and retroviruses. The D15S822 and D15S975 markers have been localized within the GABRG3 gene. The GABRG3 gene, which was previously reported as greater than 50 kb, has been shown to be approximately 250 kb. Not I and numerous Eag I restriction enzyme cut sites were identified by PFGE of clones in this region. The genomic contig of the candidate gene region will serve as a resource for the characterization of genes in individuals with AD.

Program Nr: 2607 from the 1999 ASHG Annual Meeting

Molecular basis of diabetic embryopathy: Mutation analysis of left-right axis determining genes in NOD mouse.

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As a model system to study gene-environmental interaction in the pathogenesis of birth defects, we investigated diabetic embryopathy in the offspring of symptomatic NOD [non-obese diabetic] mouse strain. The incidence of left-right axis malformations among offspring of diabetic dams varies depending on the genetic background of the fetus: When diabetic NOD dams were mated with sires of different strains, the incidence of left-right axis abnormalities is 65 and 7 percent among F1 of varies NOD x NOD, and among NOD x C57BL/6J, respectively [Morishima et al., Teratology 54:183, 1996]. We hypothesized that mutations of the key molecules involved in the normal development of left-right axis could account for the variable incidence and undertook systematic mutation analysis of NOD, and C57BL/6J. Genomic structures of murine Nodal, HNF3beta, Smad2, Acvr2b were determined by either long range PCR amplification across exons or by the rapid-amplification-of-genomic-DNA-ends method. Each exon was amplified from genomic DNA of NOD and C57BL/6J strains by PCR and the products were directly sequenced. Mutation analysis of Hnf3beta revealed a homozygous point mutation (G in C57BL/6J -> A in NOD) in the coding sequence of exon 3. This base substitution was silent but could create a splice acceptor site which would truncate the mature protein. In addition, we found 3 single nucleotide substitutions in the 5 non-coding exon L1 and a 3-base deletion -0.2kb from the start codon. These observations are compatible with the notion that DNA sequence variations in left-right axis determining genes may be associated with strain-dependent susceptibility to teratogenic effects of maternal diabetes. Furthermore, it is intriguing to note that Hnf3beta belongs to and interacts with Hnf family proteins whose mutations are associated with diabetes (MODY) in human. Therefore, mutations in Hnf3 beta could account for both situs abnormalities in the fetuses and diabetes of the dam of the NOD strain.

Program Nr: 2608 from the 1999 ASHG Annual Meeting

INVESTIGATING THE HISTAMINE 1 AND HISTAMINE 2 RECEPTOR GENES AS CANDIDATES FOR SCHIZOPHRENIA AND CLOZAPINE DRUG RESPONSE. *D.T. Mancama, J. Munro, M.J. Arranz, A. Makoff, R. Kerwin.* Psychological Medicine, Institute of Psychiatry, London, England.

There is growing evidence to suggest the involvement of histaminergic pathways in the pathophysiology of schizophrenia. The efficacy of several antipsychotic compounds is thought to include their action at histamine receptors, while overactive histaminergic activity may significantly contribute to the deficit symptoms associated with the disorder. We have selected and investigated the histamine 1 (H1) and histamine 2 (H2) receptor genes as candidates for involvement in schizophrenia and patient response to clozapine treatment. The postulated promoter and complete coding regions of the two receptor genes were screened for polymorphisms using a combination of single strand conformation polymorphism (SSCP) analysis and automated sequencing. A total of sixteen previously unpublished polymorphisms were identified, of which five were H1 receptor coding polymorphisms (Lys19Asp, Asp349Glu, 1068-A/G, Phe358D and Leu449Ser) and one an H2 receptor coding polymorphism (543-G/A). Five H1 receptor promoter polymorphisms (-17-C/T, -974-C/A, -1023-G/A, -1204-G/C, -1640-G/A) and five H2 receptor promoter polymorphisms (-294-A/G, -478-T/C, -592-A/G, -1018-G/A, -1077-G/A) were identified. Association studies using these polymorphisms revealed a marginal increase (c^2=3.94, p=0.047) in the distribution of the H1 Ser449 allele in our control sample (n=118), compared with the group of schizophrenics (n=302), though no difference was observed when the polymorphism was examined for clozapine response. None of the other H1 and H2 receptor polymorphisms were found to be associated either with schizophrenia nor with clozapine response. These results suggest that genetic variance in the H1 and H2 receptor promoter and coding regions does not significantly influence the susceptibility of individuals to schizophrenia, nor does it appear to affect response to treatment with the atypical antipsychotic clozapine.

Program Nr: 2609 from the 1999 ASHG Annual Meeting

Sequence analysis of a-**synuclein gene in familial Parkinsons disease in Finland.** *A.J. Mannermaa[1], M.J. Hiltunen[1,2], J. Autere[2], P. Jäkälä[2], K. Majamaa[3], H. Soininen[2].* 1) Chromosome & DNA Laboratory, Kuopio Univ Hosp, Kuopio, Finland; 2) Department of Neurology, Kuopio Univ Hosp, Kuopio, Finland; 3) Department of Neurology, Oulu Univ Hosp, Oulu, Finland.

The molecular background of Parkinsons disease (PD) is by far not completely understood. Recently, mutations leading to amino-acid substitutions Ala30Pro and Ala53Thr in the gene encoding a-synuclein were shown to be associated with autosomal dominant form of PD. In order to assess the possible role of a-synuclein gene mutations among the Finnish familial PD patients, we have sequenced the coding and non-coding exons of the a-synuclein gene. Thirty-six unrelated patients with typical PD and at least one affected first-degree relative were recruited from the population of Eastern Finland and Northern Ostrobothnia. The coding and non-coding exons of the a-synuclein gene were PCR amplified from genomic DNA with primers and conditions previously described. Direct sequencing of the a-synuclein gene coding exons 3-7 from the familial PD patients did not reveal mutations when compared to previously published sequences. In addition, consensus sequences at the exon-intron splice junctions were found to be intact. Sequencing of the non-coding exons 1-2, however, revealed three novel alterations in the T10A7 sequence at the 5‘end of exon 2 including insertion of A (T10A8). Other two of these alterations encompassed simultaneous appearance of insertions and deletions: T12A5 and T11A6. Same alterations were also found from the 48 age-matched control subjects indicating that the changes are not causally related to PD. However, combining the T10A8, T12A5 and T11A6 alleles into one group revealed statistically significant increase in combined allele frequencies in PD patients (0.46) when compared to control subjects (0.30): Fischers exact p = 0.05, OR 1.96 (95% CI 1.04-3.69). In conclusion, our results indicate that mutations in the coding sequence of a-synuclein gene are not responsible for familial PD in Finnish population. However, we have found three novel polymorphisms in the non-coding exon 2, which appear to be associated with increased frequency in PD patients.

Program Nr: 2610 from the 1999 ASHG Annual Meeting

Exclusion of 5-HT1B and 5-HT1D serotonin receptor genes in migraine. *P. Marttila[1], M. Kallela[2], M. Wessman[3], J. Hartiala[1], M. Färkkilä[2], J. Kaprio[4], L. Peltonen[1], A. Palotie[5].* 1) Department of Human Genetics, UCLA, Los Angeles, CA; 2) Dept. of Neurology, University of Helsinki, Helsinki, Finland; 3) Dept. of Biosciences, University of Helsinki, Helsinki, Finland; 4) Dept. of Public Health, University of Turku, Turku, Finland; 5) Dept. of Pathology and Lab. Medicine, UCLA, Los Angeles, California, USA.

Migraine is a primary episodic headache disorder having a prevalence of 10 to 20% in the general population. It has a clear genetic component, with a multigene model being most likely. Previously, gene mutations underlying a rare subtype of migraine, familial hemiplegic migraine, have been demonstrated in a calcium channel gene. A selective agonist for 5-HT1B and 5-HT1D serotonin receptors, sumatriptan, is a drug for efficient treatment of migraine. Both receptors are expressed in the human cerebral cortex and are good candidate genes for migraine pathogenesis. Both genes are coded by a single exon and are located on chromosome 6q13 and 1p34.3-36.3 respectively. In order to study these genes in patients with migraine, we have collected 250 Finnish families with familial aggregation of migraine. We chose 21 phenotypically different migraine probands from families expressing strong inheritance. The probands included individuals having migraine with aura including basilar and hemiplegic migraine cases, and migraine without aura. In addition, individuals were stratified according to their responses to sumatriptan medication. The age of onset varied between 5 and 19 years. The coding regions of both genes were sequenced in both directions from the PCR-products of the genomic DNA using an ABI-377 sequencer. We found polymorphisms in both genes, but no alterations suggestive for mutations. Thus 5-HT1D and 5-HT1B genes can be excluded as major predisposing genes for migraine, at least in the Finnish migraine population.

Program Nr: 2611 from the 1999 ASHG Annual Meeting

A very large SCA7 CAG expansion is compatible with cell viability in somatic mosaicism. *T. Matsuura[1,2], M. Khajavi[1,2], R. de Silva[3], T. Ashizawa[1,2]*. 1) Neurology, Baylor College of Medicine, Houston, TX; 2) Neurology, VA Medical Centre, Houston, TX; 3) Neurology, Institute of Neurosciences, Southern General Hospital, Scotland, UK.

Spinocerebellar ataxia type 7 (SCA7) is an autosomal dominant disorder with progressive cerebellar ataxia, macular degeneration and variable other neurological deficits including pyramidal and extrapyramidal signs, dementia, hallucinosis and propioceptive sensory loss. The disease is caused by an expansion of a CAG repeat in the ataxin 7 gene on chromosome 3p12-13. Normal alleles range from 7 to 19 repats, while the expanded vary from 37 to 306 repeats. Intermediate alleles ranging from 28 to 35 repeats that are prone to furthur expand have also been described. Expanded alleles larger than 200 CAG repeats may cause the infantile form of the disease with cardiac anomaly, hypotonia and early death (Giunti et al. Am J Hum Genet 1999, Benton et al. Neurology 1998). In sperm of patients, the expanded allele frequently exceeds 150 repeats, and relative paucity of paternally transmitted cases have been attributed to embryonic lethality due to such larger alleles (Monckton et al. Am J Hum Genet 1998;supp63). We report a 67-year-old affected female who shows somatic mosaicism of the expanded CAG repeats. The CAG repeat sizes were determined by PCR analysis as previously described. The expanded repeats are heterogeneous in size, conforming a bimodal distribution with one peak around 45 repeats and the other in the range >350 repeats. The larger peak exceeds the largest allele reported to date in the infantile SCA7 cases and shows repeat size heterogeneity. Although the age at onset was 45 years, the phenotype is severe; she has severe ataxia, complete blindness, and severe dementia with hallucinosis, chorea, myoclonus, pyramidal signs and profound propioceptive sensory loss. Our case indicates that expanded CAG repeats are highly unstable in somatic tissues in SCA7 and can exceed the putative limit of the expansion for viabile fetal development when present as mosaic alleles. The presence of alleles with >350 repeats may have contributed to the rapid progression of the disease in spite of the late onset.

Program Nr: 2612 from the 1999 ASHG Annual Meeting

A Maternally Transmitted Huntington Disease CAG Repeat Contraction from the Affected Range to the "Mutable Normal" Range. *V.L. Matthias Hagen[1], M.J. Wick[2], C. Ludowese[3], M.A. Nance[3]*. 1) Dept Med, Div Gen & Metabolism, Univ Minnesota, Minneapolis, MN; 2) Dept Lab Med and Pathol, University of Minnesota, Minneapolis, MN; 3) Huntington Disease Clinic, Hennepin County Medical Center, Minneapolis, MN.

Meiotic instability of expanded trinucleotide repeats which are the causative mutation in the triplet repeat disorders is well documented. The CAG repeats on Huntington Disease (HD) chromosomes have been shown to undergo size change in approximately 70% of meiosis,occasionally showing large expansions in paternal transmission, and usually showing minor size changes of +/- 4 repeats when maternally inherited. We have recently performed predictive HD testing on an asymptomatic individual whose mother was previously tested in our laboratory. The mother, who had no family history of HD, was affected with personality changes and movement disorder in her early 40's; molecular HD testing showed two CAG repeats of 9 and 41. This result was consistent with the clinical diagnosis of HD. Molecular analysis (performed in triplicate) of peripheral blood from the asymptomatic son showed alleles of 16 and 35 CAG repeats, indicating that the maternal allele had undergone a contraction of 6 repeats. Although alleles of repeat size 35 have not been associated with the development of HD, counseling in this case was much more circumspect. Because the son was interested in starting a family, and because it has been reported that alleles of size 27-35 demonstrate instability in sperm, he subsequently submitted a semen specimen for molecular HD analysis; the majority of sperm carrying the larger allele were found to have a repeat of size 35, with a minority containing 32-36 repeats. To our knowledge, contraction of an allele across boundaries from the abnormal range to the mutable normal range has not been previously reported. Additionally, in only a few cases has the "meiotic behavior" of alleles in the mutable normal or reduced penetrance ranges been analyzed; such analyses may be useful in the genetic counseling setting. Unusual cases such as this demonstrate the need for cautious genetic counseling of predictive testing candidates.

Program Nr: 2613 from the 1999 ASHG Annual Meeting

Genetic relationship between hypertrophic cardiomyopathy and the onset of Friedreich's ataxia. *D.O. McDaniel, C.H. Gaymes, V.V. Vedanarayanan, D.S. Braden, S.H. Subramony.* University of Mississippi Medical Center, 2500 North State Street, Jackson, MS 39216.

Hypertrophic cardiomyopathy (HCM) is a primary feature of Friedreich's ataxia (FA) disease. Considerable information has been generated as to the possible role of (GAA)n expansion in the pathogenesis of FA. To evaluate the genetic relationship between HCM and FA we studied ten patients with typical FA, six with a mild form of ataxia and age of onset between 30-70, eight with HCM, and six non-FA/non-HCM individuals. The (GAA)n repeat expansion sizes unlike typical FA in late onset of FA were not correlated with the age at onset. Among the atypical patients except one, all were heterozygous for the FA gene carrying large fragments between 700-950 repeat sizes. The homozygous patient had an intermediate expansion of approximately 136 GAA repeats which was interrupted with a GAGGAA sequence. Seven out of eight HCM patients demonstrated a homozygous expanded allele with fragment sizes between 865-950 bp.

Homozygous Alleles	Majority of Typical	Minority of Atypical	All HCM
Heterozygous Alleles	Minority of Typical	Majority of Atypical	None

A sister (46y) and a brother (70y), from two separate atypical FA patients, carried the FA gene mutation without characteristics of FA. This makes the characterization of the FA disorder more complicated. Polymorphism of the transcription factor IID (TCFIID) gene was detected in association with FA/HCM. It allowed a better stratification and correlation with disease phenotype.

Screening of HCM patients for the presence of (GAA)n mutation as well as TCFIID gene polymorphism may allow early identification of disease onset. Whether polymorphism of TCFIID gene plays a role in cardiac hypertrophy warrants further investigation.

Program Nr: 2614 from the 1999 ASHG Annual Meeting

Genomic Characterisation and Molecular Genetic Investigations on PAX4, a Candidate Gene for Silver-Russell syndrome. *S. Mergenthaler[1], K. Eggermann[1], M.B. Ranke[2], K. Zerres[1], H.A. Wollmann[2], T. Eggermann[1]*. 1) Institute of Human Genetics, Technical University, Aachen, Germany; 2) University Childrens Hospital, Tuebingen, Germany.

Silver-Russell syndrome (SRS) describes a uniform malformation syndrome characterised by intrauterine and postnatal growth retardation, asymmetry of head and limbs, a small triangular face, and other less constant abnormalities. The majority of the so far described more than 400 cases occurred sporadically, but some familiar cases indicate a genetic reason for this disease. A subset of 10% of SRS patients show maternal Uniparental Disomy for the entire chromosome 7, thus indicating the involvement of at least one imprinted gene on this chromosome. Mutations in this gene or imprinting mutations might contribute to the SRS phenotype. PAX4 as a member of a highly conserved gene family contains two DNA-binding motifs, a paired domain, and a paired-type homeodomain, which may serve its function as a transcription factor. Several genes of this family are already associated with either inherited diseases (mutations in PAX3 cause Waardenburg syndrome, PAX6 mutations lead to aniridia). PAX4 is involved in regulating developmental processes concerning the fate of pancreatic island endocrine progenitor cells, as well as the maintainance of mature beta- and delta-cell-lines in the pancreas. Mice homozygously deficient for Pax4 show growth retardation, dehydration at birth and die within 3 days. Taking into account the function of this gene, its very restricted spatial and temprorary pattern of expression during embryogenesis and its localisation on 7q32, PAX4 was considered a strong candidate gene for Silver-Russell syndrome. There is no information about the imprinting status available so far. We isolated cosmids containing fragments of human PAX4 in order to establish the genomic structure. 41 SRS-patients were subsequently screened for sequence variants, but so far, two polymorphisms have been detected, whereas no SRS-specific mutation have been found in the coding region. Investigations on parent-of-origin-specific expression in adult humans as well as in fetal tissues are currently in progress.

Program Nr: 2615 from the 1999 ASHG Annual Meeting

The HOPA gene dodecamer duplication is not a significant etiological factor in autism. *R.C. Michaelis, S.A. Copeland-Yates, K. Sossey-Alaoui, C. Skinner, M.J. Friez, J.W. Longshore, R.J. Schroer, R.E. Stevenson.* JC Self Research Inst, Greenwood Genetic Ctr, Greenwood, SC.

A recent study has suggested that a dodecamer duplication in exon 42 of the human opa-containing (HOPA) gene in Xq13 may occur in a significant portion of male patients with autism. We have determined the incidence of this duplication in 204 patients from the South Carolina Autism Project as well as control groups consisting of newborns and college students. The incidence of the duplication was not significantly different between any of our race- and sex-matched patient and control groups. In addition, there were several instances in which autism and the duplication did not cosegregate in a number of our families. Three female patients with autism inherited the duplication from nonautistic fathers. In addition, there was no systematic skewing of X inactivation in the female patients with the duplication, or in nonautistic mothers and sisters with the duplication. These findings suggest that the dodecamer duplication in the HOPA gene does not play a significant role in the etiology of autism.

Program Nr: 2616 from the 1999 ASHG Annual Meeting

Genetic factors and and high and low HDL-C level in Russians. *O.V. Miloserdova[1], P.A. Slominsky[1], V.A. Metelskaya[2], N.V. Perova[2], S.A. Limborska[1].* 1) Dept Human Molec Genetics, Inst Molecular Genetics, Moscow, Russia; 2) National Research Centre for reventive Medicine, Moscow, Russia.

We studied a possible correlation between genetic polymorphism of major apolipoprotein and lipid transport genes and total cholesterol and HDL cholesterol level in Russia population. During this work 300 male subjects (age 40-59) was screening for HDL cholesterol level and two samples of patients - with high (more than 50 mg/dl) and low (below 40 mg\dl) - was studied further according to "case-control" principle. For genetic study we analysed different kinds of DNA polymorphisms (point mutations, microsatellite and minisatellite repeats) in APOB, APOA4, APOC2, AHJC3, LHAT, CETP, LHAT, LPL genes. For biochemical study was used measurements of lipid level (total cholesterol, HDL, VLDL and LDL cholesterol) and cholesterol efflux in cell culture. Moreover, genetic and biochemical data was analysed in respect of their physical data (age, blood pressure, obesity, CHD status). However we can't find any significant influence of genetic factors to HDL cholesterol status. In Russians man sample clear differences between HDL-C attributed to non-genetic factors. Aalcohol consumption (especially strong drinks) may be one of this epigenetic factors.

Program Nr: 2617 from the 1999 ASHG Annual Meeting

Molecular characterization of a set of Portuguese patients with Friedreich ataxia. *C. Miranda[1,2], C. Barbot[3], M. Pinto[2], M.C. Moreira[2], P. Mendonça[2], J. Poirier[1], C. Ferreira[4], J. Barros[4], P. Cabral[6], J. Ferro[6], I. Silveira[2], M. Pandolfo[1], P. Coutinho[1,5], J. Sequeiros[2]*. 1) Hôpital Notre-Dame, CHUM, Montréal, Québec,Canada; 2) UnIGENe, IBMC, Univ. Porto, Porto; 3) Hosp. Maria Pia, Porto; 4) Hosp. Geral Santo António, Porto, Portugal; 5) Hosp. São Sebastião, Feira, Portugal; 6) Serv. Neurol., Hosp. Sta Maria, Lisboa, Portugal.

Friedreich ataxia (FRDA) is an autosomal recessive disorder and the most common hereditary ataxia. More than 95% of patients are homozygous for a (GAA)n expansion in intron 1 of the frataxin gene. The remaining 5% are compound heterozygotes for an expansion and a point mutation. We studied the first set of 34 Portuguese patients (28 families), who had a clinical diagnosis of FRDA. Most of these were ascertained during a systematic, population-based, survey of hereditary ataxias and spastic paraplegias, being conducted in Portugal since 1993. The geographic distribution of cases seems uniform. A (GAA)n expansion was found in 28 (82%) patients (22 families), 27 (21 families) of whom were homozygous. The mean number of GAAs was 795±151 (GAA1 allele) and 936±122 (GAA2 allele), which are within reported ranges. A few cases showed meiotic instability and the expanded allele was of the same approximate size or contracted in father-to-child transmissions, while mother-to-child transmissions had contractions or further expansions. Interestingly, one obligate heterozygote (carrying an expansion with approximately 900 GAAs), who fathered an affected child (923/1090 GAAs) had a pure cerebellar ataxia with onset at age 45. We screened exons 1 to 5a by SSCP for possible compound heterozygosity but found no point mutations; we also excluded CAG expansions in all known genes for dominant ataxia (SCA1-3, 6-8, DRPLA). This could either be a manifesting heterozygote for FRDA (which has never been described), a phenocopy, or the result of an unknown mutation, e.g. in yet another gene for dominant ataxia. We cannot exclude, however, a mutation that escaped SSCP analysis or lies outside the coding region. The gene will be sequenced to answer this question.

Program Nr: 2618 from the 1999 ASHG Annual Meeting

Towards the isolation of differentially expressed mRNAs from the FMR1 knockout mouse. *J. Morales, D.L. Nelson.* Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX.

Fragile X Syndrome is the most common cause of inherited mental retardation. The majority of cases are caused by the expansion and methylation of a CGG repeat in the 5' UTR of the FMR1 gene resulting in the silencing of the gene. The FMR1 protein is expressed in a variety of tissues with high levels in the hippocampus, cerebellum and testis. The FMR1 KO mouse exhibits a phenotype similar to that of humans. Analysis of the FMRP sequence revealed the existence of several functional RNA binding domains. It has been reported that FMRP is capable of binding RNA in vitro suggesting that RNA binding and regulation may be its primary function. Thus the identification of RNAs capable of interacting with FMRP is essential to the understanding of the pathway in which it works.

The isolation of RNAs that are affected in the absence of FMRP has been the primary focus of this project. Two different approaches have been undertaken in order to compare the RNA population of the knockout mouse to that of the wild type litter mate. A PCR based cDNA subtraction scheme was employed to analyze differences in whole brain, testis and hippocampus. A total of 28 clones were selected for further study. Of these, 8 had been reported previously in the database and 20 were unknown. RT-PCR and Northern blots were performed to confirm the results of the subtraction. None of the clones however, showed an observable difference between wildtype and knockout cells. In order to clarify our observations and refine our hypothesis, a cDNA hybridization approach has been initiated. Genome Systems cDNA arrays have been hybridized with cDNAs generated from hippocampus of knockout and wild type mice. Our preliminary data shows that 4 clones (neuroleukin and 3 unknown) are >30 fold higher in the knockout whereas 3 clones (asialoglycoprotein receptor and 2 unknown) are >20 fold higher in the wild type. A number of clones that display subtle differences in RNA levels are also being analyzed. RT-PCR and Northern blots are currently being performed to verify our results. The cDNA arrays will also be utilized to look at differences in the RNA population of testis and cerebellum.

Program Nr: 2619 from the 1999 ASHG Annual Meeting

Genetic analysis of Candidate genes in Nonsyndromic Cleft Lip with or without Cleft Palate Families. *L. Moreno[1], M. Arcos-Burgos[2], M. Marazita[3], K. Neal[1], B.S. Maher[3], C. Valencia[4], I. Fonseca[2], A.C. Lidral[1]*. 1) Orthodontics, Ohio State University, Columbus, OH; 2) Department of Biology, University of Antioquia, Medellin, Colombia, SA; 3) Cleft Palate-Craniofacial Center and Dept of Human Genetics, University of Pittsburgh; 4) College of Dentistry, University of Antioquia, Medellin, Colombia, SA.

Nonsyndromic cleft lip with or without cleft palate (CL/P) is a common, genetically complex, birth defect. Previous studies using candidate gene strategies to identify the genetic basis for Cl/P have revealed significant results for TGFA, TGFB3, and MSX1. In addition, translocations in the regions of 19q13 and 6p24 are associated with CL/P phenotypes and evidence from linkage and linkage disequilibrium studies support this association. However, studies of these candidate genes and loci have been contradictory, suggesting that heterogeneity between study populations may exist. The purpose of this study is to extend the evaluation of these candidate genes in multiplex nonsyndromic CL/P families from Central Ohio and Medellin, Colombia, SA. Twelve Ohio and 35 Colombian families were analyzed using parametric (dominant and recessive models were used in Linkage and GENEHUNTER) and nonparametric (GENEHUNTERnpl and SimIBD) linkage approaches. In addition, heterogeneity was analyzed using GENEHUNTER. Preliminary analysis of TGFA and TGFB3 revealed no evidence for linkage with TGFB3. However, SimIBD analysis showed suggestive linkage to the TGFA marker D2S443 (p=0.077), indicating that TGFA may play a role in the etiology of CL/P in these populations. Efforts are ongoing to genotype more markers for these and additional candidate genes to determine whether they have a role in CL/P in these populations. In addition, heterogeneity is being evaluated within these families and populations.

Program Nr: 2620 from the 1999 ASHG Annual Meeting

Linkage Disequilibrium Mapping In Reading Disability. *D.W. Morris[1], D. Turic[1], L. Robinson[1], M. Duke[1], V. Webb[1], M. Easton[1], S. Fernando[1], K. Pound[1], J. Stephenson[2], P. McGuffin[3], M.J. Owen[1], M.C. O'Donovan[1], J. Williams[1]*. 1) Department of Psychological Medicine, University of Wales College of Medicine, Heath Park, Cardiff, UK, CF14 4XN; 2) Psychology Department, University of Southampton, Southampton, UK, S017 1BJ; 3) SGDP Research Center, Institute of Psychiatry, London, SE5 8AF.

Developmental dyslexia/reading disability is a common condition with established heritable components. Replicated linkage analyses have highlighted two chromosomal regions which show suggestive evidence of susceptibility genes. We have collected a sample of over 100 unrelated probands and their parents for family based linkage disequilibrium mapping. Participants were collected from the population of South Wales (UK). Probands (aged 7 - 17 years) were defined as having a normal IQ (85 or above) with a general reading age at least 2.5 years below age-related norms. Linkage disequilibrium studies using polymorphic microsatellite markers were carried out on the 2 chromosomal regions of putative linkage. Evidence for allelic association was observed in both regions for markers in both regions (chromosome 6p21; p=0.01, chromosome 15q21; p=0.0015). These results were further supported by haplotype analyses using surrounding markers. Our data provide further support for susceptibility genes for dyslexia on chromosomes 6 and 15, and suggest more refined map positions of these loci. Replication and finer scale mapping is currently underway.

Program Nr: 2621 from the 1999 ASHG Annual Meeting

Further analysis of the association of tau with progressive supranuclear palsy. *H.R. Morris[1,2], M. Baker[2], A.J. Lees[3], M. Hutton[2], J. Hardy[2], N.W. Wood[1].* 1) Neurogenetics, Institute of Neurology, London, UK; 2) Neurogenetics Laboratory, Mayo Clinic, Jacksonville, Florida, USA; 3) National Hospital for Neurology, Queen Square, London UK.

Progressive supranuclear palsy (PSP) is a neurodegenerative condition characterised by the deposition of tau protein containing neurofibrillary tangles. The A0 allele of an intronic microsatellite marker of the tau gene is positively associated with the development of PSP. We have undertaken further analysis of six intragenic single nucleotide polymorphisms (SNPs) which span 100 kb at the tau locus, and five microsatellite markers in a 4.5 cM region around the tau gene. We have confirmed that the common variant at each intragenic SNP is associated with the development of PSP and that the SNPs are inherited together in unrelated Caucasian individuals as two major tau haplotypes. Analysis of the microsatellite marker allele distribution using the Likelihood Ratio Test shows that none of these close flanking markers are associated with the development of PSP ($LRT<1.2$, $p>0.15$). This refines the limits of the association of the immediate tau region with the development of PSP, and the extent of linkage disequilibrium around tau in the general population. It does not support the presence of a widely distributed pathogenic mutation ocurring on a common tau haplotype background, nor the involvement of genes other than the most likely candidate, tau.

Program Nr: 2622 from the 1999 ASHG Annual Meeting

Frequent duplications of triplet interruptions within a seven generation SCA8 family. *M.L. Moseley, M.D. Koob, L.P.W. Ranum.* Depts of Genetics, Cell Biology and Development, Neurology, and Institute of Human Genetics, University of Minnesota, Minneapolis, MN.

We recently described a novel CTG expansion that causes a previously undescribed ataxia (SCA8). In contrast to other triplet repeat diseases, pathogenic CTG expansions can have hypermutable sequence interruptions. The SCA8 CTG repeat is preceded by a polymorphic but stable CTA tract, with the configuration $(CTA)_{3\text{-}17}(CTG)_n$. The CTG repeat tract is elongated on pathogenic alleles and nearly always changes in size when transmitted from one generation to the next. Sequence interruptions observed within expanded CTG tracts among 11 ataxia families include: CCG, CTA, CTC, CCA and CTT. These interruptions, clustered at the 5' end of the repeat tract, do not appear to affect penetrance as both interrupted and uninterrupted alleles are found in affected individuals.

We are studying a seven-generation ataxia family that has CCG triplet interruptions within the CTG tract. Although haplotype analysis confirms that the expanded alleles are from a common founder, the CCG interruptions were often duplicated resulting in six different allele configurations. The presence of uninterrupted CTG expansions in one branch of the family suggests that a least one transmission within the past seven generations resulted in either the addition or deletion of a CCG interruption. In two instances duplications of the CCG repeat occurred over a single generation: $(CTG)_5\mathbf{CCG}(CTG)_{101}$ changed to $(CTG)_5\mathbf{CCG}(CTG)_7\mathbf{CCG}(CTG)_{105}$, while $(CTG)_5\mathbf{(CCG)_3}(CTG)_{103}$ became $(CTG)_5\mathbf{(CCG)_4}(CTG)_{87}$. In other instances repeat duplications that occurred in particular family branches could be inferred. These frequent mutation events altered the CTG repeat configuration at least six times in 28 transmissions. The presence of interruptions within the pathogenic expansion and their extremely high mutation rate are not observed in other triplet diseases.

Program Nr: 2623 from the 1999 ASHG Annual Meeting

Human mitochondrial ribosomal protein gene L12: A candidate gene for Russell-Silver syndrome. *E.B. Mougey[1], B.A. Maguire[1], J. Liu[2], J.E. Sylvester[1], T.W. O'Brien[2].* 1) Cellular and Molecular Medicine, Nemours Children's Clinic, Jacksonville, FL; 2) University of Florida, Gainesville, FL.

Mammalian mitochondrial ribosomes are responsible for translation of 13 mitochondrially encoded mRNAs that code for components of the oxidative phosphorylation system. The goal of the Human Mitochondrial Ribosomal Protein Consortium is to clone, map and study human mitochondrial ribosomal protein genes (hMRPs), (see abstract, Sylvester, et al., this meeting). It is well known that defects in other mitochondrially encoded components of the mitochondrial translation system, i.e. tRNAs, and rRNAs lead to human disease. Based on these observations, we hypothesize that mutations in hMRPs will also lead to human disease. To test this hypothesis, candidate diseases will be identified from the expanding list of mapped human disorders by using hMRP map location and genomic sequence generated by the consortium. The first hMRP gene that we are focusing on is the human homologue of bacterial L7/L12 (hMRP L12). hMRP L12 is critical for the function of several elongation factors and as such mutations in hMRP L12 should not be well tolerated. hMRP L12 has been mapped to 17q25 by FISH (Marty et al., Genomics 1;41(3):453-7, 1997; J. Liu, B. Barnoski and T. OBrien) and Ramirez-Duenas, et al., 41:51-53, 1992, and Midro, et al., Clin.Genet. 44:53-55, 1993, have described Russell-Silver patients with 17q25 translocations t(17;20) (q25;q13) and t(1;17) (q31;q25), respectively. The two main clinical features of Russell-Silver Syndrome are lateral-asymmetry and low birth weight dwarfism, i.e. retarded and abnormal development, both characteristics are consistent with reduced mitochondrial function. We present a strategy to identify hMRP L12 mutations in Russell-Silver patients based on PCR amplification of the entire hMRP L12 genomic region coupled with sequencing, and RT/PCR analysis of hMRP L12 mRNA coupled with sequencing. The strategy presented will be useful for the analysis of all mapped hMRP genes that are potential factors in human pathology.

Program Nr: 2624 from the 1999 ASHG Annual Meeting

Adult ADHD: association with the dopamine D4 receptor gene. *P. Muglia[1], U. Jain[2], V.S. Basile[1], J.L. Kennedy[1]*. 1) Neurogenetics Section,; 2) Adult ADHD Clinic, Department of Psychiatry, Centre for Addiction and Mental Health, University of Toronto, Toronto, Ontario, Canada.

The Attention deficit hyperactivity disorder (ADHD) is the most common child psychiatric disorder characterized by the combination of developmentally inappropriate attention, hyperactivity, and impulsivity. One third of the ADHD children continue to suffer from the disorder in adult life. A higher genetic loading for adult is suggested by findings of a higher relative risk in adults vs. childhood phenotypes (19 vs. 5, respectively), as ascertained by family studies. This increases the statistical power of genetic studies examining adult ADHD. Evidence indicates the dopamine system as primary candidate in the pathophysiology of ADHD and the dopamine D4 receptor gene (DRD4) represents a promising candidate. The third exon of DRD4 contains a 48-bp tandem repeat that can occur 1 to 10 times in the population. Each of these tandem repeats code for a 16 amino acid sequence that constitute the intracytoplasmic loop of the receptor. In vitro studies have shown that the 7-repeat allele of DRD4 has a small but significant blunted transmission of the signal if compared to the others alleles. Following our positive association of the 7-repeat allele in ADHD in children, various groups have replicated the result in independent samples. We genotyped the VNTR polymorphism of DRD4 in 33 adult ADHD and in 33 controls. Genotype analysis in the case control sample demonstrates that the 7-repeat allele is significantly more common in adult ADHD than in controls ($c2 = 7.16$, df=2, p=0.027). In an allelic analysis comparing the 7, 4 and the other repeat alleles, we also found significant results ($c2 = 6.80$, df=2, p=0.03). These results even if in a small sample confirm the significant higher presence of the 7 allele in ADHD children also in the adult subjects. The genotyping in a separate sample of trios is in progress.

Program Nr: 2625 from the 1999 ASHG Annual Meeting

Characterization of DNA methylation patterns in the 5' region of necdin, an imprinted gene in the Prader-Willi Syndrome region. *M.L. Munn, R. Wevrick.* Medical Genetics, University of Alberta, Edmonton, Alberta, Canada.

Prader-Willi Syndrome (PWS) is a neurobehavioural disorder which affects about 1/15000 people. Patients exhibit severe hypotonia and failure to thrive in infancy followed by obesity and developmental delay. PWS patients usually have either deletions in their paternal chromosome 15 (~70%) or maternal uniparental disomy for chromosome 15 (~30%). Since the phenotype is dependent on parent of origin, genomic imprinting is implicated in this disorder. *NDN*, which encodes necdin, a protein involved in terminal differentiation of neurons, is one of four imprinted genes found in the PWS critical region. Methylation of CpG dinucleotides is one proposed mechanism for imprinting of specific genes. One site of maternal allele-specific methylation has already been detected by methylation-sensitive restriction digest analysis of EagI sites in the human and mouse necdin genes. In order to understand how the primary imprinting mark is established, we are characterizing in detail the CpG methylation patterns in the 5' region of *Ndn* (the mouse homologue of *NDN*) using the bisulfite-sequencing method in different adult tissues and early developmental stages. We can recognize allele-specific differences in DNA methylation using tissues from F1 crosses between different mouse species that exhibit DNA polymorphisms in this region. We are currently using mouse brain (expressing), liver (non-expressing) and heart (non-expressing) adult tissues. Our initial results include analysis of 39 contiguous CpG dinucleotides. They suggest that the maternal allele is more methylated than the paternal allele in brain, but in liver, both alleles are relatively unmethylated. We have collected preimplantation embryos from 2 cell, 4 cell, 8 cell and blastocysts and are also analyzing embryonic stem cells, sperm and oocytes. Preliminary results from the developmental analysis suggest the presence of an oocyte-specific methylation pattern which may serve as a gametic imprint for the *Ndn* gene. These experiments will provide the first insight into how genes are turned on or off in an allele-specific manner.

Program Nr: 2626 from the 1999 ASHG Annual Meeting

No association of the G-protein b**3 subunit C825T polymorphism with essential hypertension in an African Caribbean population.** *P.B. Munroe[1], D. Jadhav[1], M. Sandhu[1], J. Knight[1], A.J.L. Clark[2], M.J. Caulfield[1]*. 1) Clinical Pharmacology, St. Bartholomew's Hospital, London, United Kingdom; 2) Chemical Endocrinology, St. Bartholomew's Hospital, London, United Kingdom.

Essential hypertension and its complications, coronary heart disease, stroke and renal failure are leading causes of morbidity and mortality in westernised societies. The cellular sodium-hydrogen exchanger (NHE-1) is reported as having increased activity in up to 50% of hypertensive individuals, and recent data suggests a polymorphism, C825T, in the G-protein b3 subunit (GNB3) might account for this increased NHE-1 activity via enhanced cellular signal transduction. Studies of the C825T polymorphism have also revealed an association with essential hypertension in Caucasian individuals. A case-control study involving 221 hypertensive and 91normotensive African Caribbean individuals from St. Vincent and the Grenadines was performed to test for association between the C825T variant and essential hypertension. Analysis of the polymorphism was performed with the polymerase chain reaction and restriction digestion using *BseDI*. No association was found between the C825T polymorphism and essential hypertension ($c2 = 1.091$, 2df, $p = 0.58$), however, the frequency of the T allele was exceptionally high in this population (0.75 as compared to 0.30 in Caucasians).These results generate the hypothesis that GNB3/NHE-1 activity may be increased in this African Caribbean population and thus functional studies are warranted in this ethnic group.

Program Nr: 2627 from the 1999 ASHG Annual Meeting

A PNA-clamping based method of detection of point mutations in mtDNA. *D.G. Murdock, N. Christacos, S. Melov, D.C. Wallace.* Ctr Molec Med, Emory Univ, Atlanta, GA.

Inherited mitochondrial DNA (mtDNA) mutations have been shown to cause a variety of degenerative diseases, and the accumulation of somatic mtDNA mutations have been implicated in aging. Detection of mutations in mtDNA is complicated by the fact that each cell contains hundreds to thousands of mtDNAs, and a mutant cell can contain any proportion of mutant or wild type molecules, a condition known as heteroplasmy. As a result, it is often important to be able to detect low proportions of mutant mtDNAs in maternal relatives at risk for a disease or in the tissues of aging individuals. To develop a reliable procedure for detecting such mutations with low heteroplasmy, we have applied the peptide nucleic acid (PNA) clamping PCR technique to two pathogenic point mutations: the tRNA-Leu np3243 mutation causing mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes (MELAS), and the tRNA-Lys np8344 mutation causing myoclonic epilepsy and ragged red muscle fibers (MERRF). We have shown that the PNAs can selectively block amplification of the wild type molecule, leaving the mutant molecules available for amplification, and that the reaction can be multiplexed to identify more than one mutation per reaction. This method is extremely sensitive, being capable of detecting mutations at the level of 0.1% of total molecules, without false positives in the absence of mutant molecules. With this highly sensitive and specific assay, it will now be possible to evaluate the levels of somatic mtDNA point mutations in aging tissue.

Program Nr: 2628 from the 1999 ASHG Annual Meeting

Ethanol Responsive Genes Contributing To Alcoholism Identified by cDNA Expression Arrays. *B.C. Murphy, T. Chiu, P. McDonald, S.M. Singh.* Molecular Genetics Unit, Western Science Centre, University of Western Ontario, London, Ontario, Canada N6A 5B7.

Genetic strains of mice with extreme voluntary alcohol consumption (VAC) and sensitivity remain an invaluable model for the identification of genes involved in the complex etiology of alcohol preference leading to alcoholism. Ethanol has a direct effect on gene expression that is gene and strain/genotype specific. Variable expression of strain specific ethanol responsive (SSER) genes associated with VAC represent a logical approach to the identification of genes contributing to alcoholism.

Sequencing of many genes have prompted the development of the novel but untested expression array technology. We used Clontech's mouse cDNA expression array, containing 588 known genes. We labeled cDNA representing brain and liver of two strains (C57BL/6J with high and BALB/c with low VAC) that were ethanol fed using isocaloric liquid diet with matched controls. Expression differences between control and experimental animals were visually apparent and confirmed by computer analysis. In the liver, fifteen C57BL/6J and seven BALB/c genes were upregulated while none of the genes in either strain were downregulated in response to ethanol. Upregulation of six of these genes was observed in both strains. A number of these have been confirmed by semi-quantitative RT-PCR. Unlike the liver, brain specific upregulation of genes was confined only to the C57BL/6J strain. The single common gene upregulated in the two tissues was identified as glutathione S-tranferase. A number of other genes identified in this research on expression arrays are compatible with their known function. Others still under characterization may provide genes of importance in the complex etiology of alcohol preference. These results support the value of expression arrays in the identification of important genes in most complex etiologies.

Supported by Medical Research Council and the Ontario Mental Health Foundation.

Program Nr: 2629 from the 1999 ASHG Annual Meeting

Identification of QBP1, a synthetic peptide that preferentially binds expanded polyglutamine domain by phage display technique. *Y. Nagai[1,3], T.J. Tucker[1,3], D.J. Kenan[4,5], B.S. Henderson[4], J.D. Keene[4], W.J. Strittmatter[1,2,3], J.R. Burke[1,3]*. 1) Medicine (Neurology); 2) Neurobiology; 3) Deane Laboratory; 4) Microbiology; 5) Pathology, Duke University Medical Center, Durham, NC.

Expansion of polyglutamine domain is responsible for eight inherited neurodegenerative diseases, including Huntingtons disease, dentatorubral pallidoluysian atrophy (DRPLA), spinobulbar muscular atrophy (SBMA), and spinocerebellar ataxias (SCA1,2,3,6,7). The length of the polyglutamine domain is critical to pathogenesis and determines age of disease onset and disease severity. The mechanism by which expanded polyglutamine domain causes neurodegeneration remains unclear, but may be due to altered conformation leading to pathogenic protein-protein interactions. Molecules that selectively bind expanded polyglutamine domains may inhibit such pathogenic protein-protein interactions, and may be useful as a therapeutic tool. To identify a peptide that preferentially binds expanded polyglutamine domain, we screened a combinatorial M13 phage display library for binding to 19 or 62 glutamines (Q19 or Q62)-glutathione-S-transferase (GST) fusion proteins. We identified a peptide, named QBP1 (Glutamine Binding Peptide 1), which preferentially binds Q62-GST fusion proteins as compared to Q19-GST. Co-expression of Q81-yellow fluorescent protein (YFP) and QBP1-cyan fluorescent protein (CFP) fusion proteins in transfected COS-7 cells demonstrated co-localization of QBP1-CFP into the Q81-YFP aggregates. Our observations suggest that QBP1 fusion protein can be incorporated into polyglutamine aggregate and may be useful for developing potential therapies.

Program Nr: 2630 from the 1999 ASHG Annual Meeting

Investigation of TCF19 as a candidate gene for psoriasis susceptibility. *R.P. Nair[1], N.V. Chia[1], S. Jenisch[2], P. Stuart[1], J. Epperson[1], T. Henseler[3], E. Westphal[2], J.L. Arndt[1], E. Christophers[3], J.J. Voorhees[1], J.T. Elder[1,4]*. 1) Dept Dermatology, Univ Michigan, Ann Arbor, MI; 2) Dept Immunology, Univ Kiel, Germany; 3) Dept Dermatology, Univ Kiel, Germany; 4) Ann Arbor Veterans Affairs Hospital, Ann Arbor, MI.

Genome-wide searches for psoriasis susceptibility genes identified candidate regions on chromosomes 1q, 4q, 6p, 16q, 17q and 20p. The chromosome 6 locus (PSORS1, 6p21.3) encompasses HLA Class I and II genes long known to be associated with psoriasis, and linkage has been now been replicated in several family studies. Association analysis using the transmission / disequilibrium test (TDT) narrowed the susceptibility region to a ~300 kb interval in the centromeric HLA Class I region containing the HLA-B and -C genes. However, HLA haplotypes in this interval are in stronger linkage disequilibrium than are their component alleles, suggesting that the causative gene is probably not HLA-B or HLA-C. Transcription factor gene TCF19 (SC1) maps to this interval and is therefore a candidate for psoriasis susceptibility. We investigated polymorphisms in the exons of TCF19 in 4 heterozygous psoriasis patients carrying the two most common HLA susceptibility haplotypes (B57-Cw6 and B13-Cw6). One nondiseased B7-Cw7 homozygote served as a control, because this common haplotype has not been associated with psoriasis in Caucasians. Unlike the HLA-B and HLA-C genes centromerically and the corneodesmosin gene telomerically, TCF19 did not appear to be polymorphic. In the seven chromosomes examined we found no variations when compared to the published cDNA sequence (Genbank ID U25826), other than two single base insertions present in all chromosomes examined (3989insG and 4162insA). These variations are in agreement with the recently published genomic sequence of the region (Genbank IDs AC004195 and AC006047). These insertions would alter the predicted protein sequence and shorten it by 15 amino acids. Based on these results, we conclude that TCF19 is unlikely to be involved in psoriasis. Moreover, our results indicate that the distribution of allelic polymorphism is not uniform across centromeric HLA Class I.

Program Nr: 2631 from the 1999 ASHG Annual Meeting

The expression of genes important in endochondral ossification in human osteoarthritic tissue. *W.G Newman[1], K.L Chapman[1], M.C Hillarby[2], A.J Freemont[2], M.E Grant[1], R.P Boot-Handford[1], G.A Wallis[1].* 1) Wellcome Trust Centre for Cell Matrix Research, University of Manchester, Manchester,M13 9PT, UK; 2) Department of Pathological Sciences, University of Manchester, Manchester M13 9PT, UK.

Osteoarthritis (OA) is a common, complex disorder, characterised by cartilage loss and new bone formation at synovial joints. Recent work suggests that the process which forms the bony skeleton, endochondral ossification (EO), is reactivated in the development of OA. The new bone growth is related to disease progression and may be the initiating event in OA pathogenesis. We hypothesised that genes that are differentially expressed by chondrocytes during EO may have a role in the initiation and progression of OA.

To test this theory, an RT-PCR subtractive hybridisation was performed to create a cDNA pool enriched for proliferative chondrocyte cDNAs. The cDNA pool was validated and used to screen a foetal bovine chondrocyte lZap cDNA library. Six genes were identified which encode alpha enolase, heavy chain ferritin subunit, annexin V, matrix Gla protein, nexin and a vitamin D responsive transcript. The differential expression of these genes in the bovine growth plate has been confirmed by northern blot analysis and immunohistochemistry. Preliminary results suggest that the genes differentially expressed by the proliferative chondrocytes are up-regulated in OA tissue compared to normal human articular cartilage and as such may provide important clues regarding the pathogenesis of this disease.

Program Nr: 2632 from the 1999 ASHG Annual Meeting

Instability of FMR1 alleles with 40-60 CGG repeats. *S.L. Nolin, G.E. Houck, Jr., A.D. Gargano, S.Y. Li, A. Glicksman, W.T. Brown.* Institute for Basic Research, Staten Island, NY.

The stability of intermediate fragile X alleles with 40-60 CGG repeats is uncertain. To assess the frequency of instability, we have analyzed 92 transmissions in 60 families with no known history of fragile X. In families with repeats between 40-44, no unstable transmissions were observed in 32 meioses. No instabilities were observed among 16 meioses in 10 families with repeats from 45-49. One additional family, referred to us because of an instability, exhibited an increase of two repeats from 45 to 47 in three of five daughters. In the 50-54 repeat range, 3 of 25 (12%) meiotic events were unstable. Two of the three, however, were contractions and only one was an expansion. In the 55-59 repeat range, 5 of 20 (25%) meioses expanded by a few repeats, and 15 (75%) inherited the allele without change. These data suggest that instabilities in the 40-49 range are infrequent and that the risk of expansion to the full mutation in one transmission is negligible. Alleles in the 50-54 range have some propensity for expansion, but the risk for expansion to the full mutation in one transmission is low. Alleles >55 are likely to carry higher risks for expansion.

For 14 males with repeats 50, AGG interruptions were analyzed by sequencing to compare the length of pure CGG repeats with repeat instability. No unstable transmissions were observed in six families with <40 pure CGGs. In 3 males from families with unstable transmissions, 41, 42, and 49 pure CGGs tracts were observed at the 3' end. Five other families with 40-49 pure CGGs failed to exhibit repeat instability. These results indicate that the presence of pure CGG tracts >40 does not always confer instability on alleles in this size category and that additional factors are likely to influence instability. ’

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Program Nr: 2633 from the 1999 ASHG Annual Meeting

Amino acids flanking polyglutamine stretches influence the competence of aggregate formation. *K. Nozaki, O. Onodera, S. Tsuji.* Dept. of Neurology, Brain Research Institute, Niigata University, Niigata, Japan.

Background: Expanded polyglutamine stretches have been shown to form aggregates and to be toxic to cells. Interestingly, however, the threshold of the size of expanded CAG repeats vary among the diseases caused by CAG repeat expansions. We hypothesized that amino acids flanking the polyglutamine stretches substantially influence the competence of aggregate formation of the mutant proteins, and eventually the clinical presentations in diseases caused by expansions of CAG repeats.

Method: Partial cDNA fragments containing various lengths of CAG repeats, and the flanking fragments (24-bp upstream fragment and 27-bp downstream fragment) for SCA2, HD, DRPLA, and MJD/SCA3 were cloned into pEGFPN1 to allow expression of polyglutamine stretches fused with GFP. These plasmids were transfected into COS-7 cells, and the frequencies of cells with aggregates were determined for each construct 72 hours after transfection.

Results: In the COS cells transfected with the SCA2 and DRPLA cDNA fragments, aggregate formation rate increases in a CAG repeat length-dependent manner with threshold between 34 and 36 repeats (ataxin-2; 6.5±1.6%®20.1±4.4%, DRPLAP; 7.5±3.7%®32.5±6.1%). When the rate of aggregate formation were compared among ataxin-2, huntingtin, DRPLAP and ataxin-3 containing the same size of CAG repeats (56 repeats), ataxin-2 (66.5±4.4%) and huntingtin (60.7±4.4%) showed significantly higher aggregate formation rate than DRPLAP (39.1±6.1%) and ataxin-3 (30.3±5.3%). These results are in good agreement with the fact that the thresholds of expanded CAG repeats in DRPLA and MJD patients are much larger than those in HD and SCA2 patients.

Conclusion: These results indicate that amino acids flanking the CAG repeats substantially influence the competence of aggregate formation.

Program Nr: 2634 from the 1999 ASHG Annual Meeting

Nuclear Localization of Truncated Atrophin-1, The DRPLA Gene Product, Increases Cellular Toxicity. *F.C. Nucifora[1], W.J. Herring[2], M.F. Peters[1], J.D. Wood[1], R.L. Margolis[1], V.L. Dawson[2], T.M. Dawson[2], C.A. Ross[1].* 1) Division of Neurobiology, Dept. of Psychiatry, Johns Hopkins School of Medicine, Baltimore, MD; 2) Dept. of Neurology, Johns Hopkins School of Medicine, Baltimore, MD.

Dentatorubral and pallidoluysian atrophy (DRPLA) is a neurodegenerative disorder similar to Huntingtons disease, with chorea, incoordination, ataxia, and dementia caused by an expansion of a CAG repeat encoding polyglutamine in the atrophin-1 gene. Identification of an N-terminal fragment of atrophin-1 in patients and transgenic mice containing 65 glutamine repeats suggests that truncation plays a role in the disease process. In transient cell transfection experiments using N2a cells, full length atrophin-1 with 26 or 65 glutamine repeats shows granular nuclear and cytoplasmic staining with no increase in toxicity caused by the expanded repeat. Atrophin-1 contains a putative bipartite nuclear localization signal (RKKEAPGPREELRSRGR) near the N-terminus (a.a. 16) and a putative nuclear export signal (LARLQMLNV) near the C-terminus (a.a. 1033). Constructs lacking the putative NES (a.a. 1-917, At-N917), show an increase in diffuse nuclear labeling and an increased toxicity (36.7%) for this construct with 65 repeats compared to this construct with 26 repeats (19.4%). Mutating the NLS (NNKEAPGPREELNSRGN) in At-N917-26Q or At-N917-65Q shifted atrophin-1 to the cytoplasm and eliminated the enhanced toxicity of 65 repeats. When the putative NES was mutated (AARAQMANV) full length atrophin-1 showed mostly diffuse nuclear staining and the expanded repeat now caused cell toxicity comparable to that of the truncation (36.5%). These data indicate that endogenous cellular targeting signals are functional, that the effect of truncation is to delete the NES, and that nuclear localization is directly related to cell toxicity . They are also consistent with the idea that proteolytic processing is relevant for toxicity. This work was supported by the HDSA, NS16375, and NS38377.

Program Nr: 2635 from the 1999 ASHG Annual Meeting

Stability of GAA•TTC triplet repeats in transgenic mice. *K. Ohshima, J. Poirier, M. Pandolfo.* CHUM, Campus Notre-Dame, Montreal, PQ, Canada.

Meiotic and mitotic instabilities of GAA•TTC triplet repeats are characteristic of Friedreich ataxia in which about 100 to more than 1,000 GAA•TTC triplets are found in the first intron of the target frataxin gene. We generated transgenic mice carrying about 250 GAA•TTC triplets. The repeats were cloned in both orientations within the intron of a reporter gene in a pSPL3-derived construct whose expression is controlled by the SV40 promoter. The DNA constructs were injected into the oocyte pronuclei of fertilized C57BL6/C3H female mice. Pups were evaluated for the presence of the transgene by Southern blot and PCR analyses on DNA extracted from tail blood samples. Seven transgenic lines were obtained for the construct in which TTC strand is on the transcript. We determined the size of repeats in the founders and their progenies (F1 and F2 generations) by PCR and Southern blot analyses. Most lines harbor multiple copies of the transgene, in some of which the GAA•TTC repeat underwent size changes (mostly contractions) at the time of insertion into the mouse genome. However, the repeats subsequently remained stable during meiotic and mitotic transmissions with no detectable increases or decreases in size regardless of orientation . We conclude that GAA•TTC triplet repeats containing up to 250 triplets are stably transmitted in the mouse at least in the context of the DNA construct we used to generate transgenic animals. In human, repeats of similar length contained in the first intron of the frataxin gene show moderate meiotic instability.

Program Nr: 2636 from the 1999 ASHG Annual Meeting

Interleukin-4 receptor alpha chain polymorphism Gln551Arg is associated with adult atopic dermatitis in Japan.
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Localization of a locus for atopy to chromosome 16p12-p11 and reported associations of Ile50Val and Gln551Arg polymorphisms in IL4R gene with atopy prompted us to sequence the gene in 27 adult atopic dermatitis (AD) and 29 non-atopic (non-AD) subjects. All six IL4R missense polymorphisms and a silent L389L (CTG>CTT) polymorphism were clearly detected by sequencing. There were no statistically significant differences in codon 50 genotypes between AD and non-AD. The most significant association was found for the codon 551 polymorphism (p=0.010). Six of 27 atopic dermatitis patients were heterozygous for the Arg551 allele, but this allele did not occur in the 28 control subjects. The disease severity among the six Arg551 heterozygous AD subjects was variable; two had a mild phenotype and four had a severe phenotype. Ser411Leu and Ser761Pro polymorphisms were not detected in this study. One of 26 atopic subjects and three of 28 normal subjects were heterozygous for the Ala375 allele, which is not statistically significant. All of the four individuals who were heterozygous for Ala375 were heterozygous for both Arg406 and Leu389Leu (CTG>CTT), suggesting that these three polymorphisms probably occur in the same haplotype. However, this haplotype had no association with the development of AD.Very recently, Noguchi et al studied Glu551Arg polymorphism of IL4R gene in Japanese asthmatics, and found no association with atopy. The frequency of the Arg551 allele in their study was 0.16 in atopic subjects versus 0.13 in non-atopic parents. In our study, the frequency of the Arg551 allele was 0.11 in AD versus 0.00 in non-AD. The higher allele frequency of Arg551 allele in the non-atopic group in Noguchis report may reflect relatively low penetrance of this allele in the development of asthma among non-atopic parents. The reason for the low allele frequency of Arg551 in the present study may be that we excluded subjects as controls if they had a family history of any atopic diseases, and therefore we were able to exclude non-penetrant cases. In conclusion, the IL4R gene should be considered a strong candidate gene for AD.

Program Nr: 2637 from the 1999 ASHG Annual Meeting

Analysis of cell toxicity on cultured cells mediated by the a**1A-voltage-dependent calcium channel gene with CAG repeat expansion (SCA6 gene).** *K. Owada[1,3], K. Ishikawa[1,3], H. Saegusa[2,3], T. Tanabe[2,3], H. Mizusawa[1,3].* 1) Neurology, Tokyo Medical & Dental Univ., Tokyo, Tokyo, Japan; 2) Pharmacology, Tokyo Medical & Dental Univ., Tokyo, Tokyo, Japan; 3) CREST, Japan Science and Technology Corporation, Saitama, Japan.

Spinocerebellar ataxia type 6 (SCA6) is a late-onset neurodegenerative disorder caused by expansion of trinucleotide (CAG) repeat in the 3' region of the a1A-voltage-dependent calcium channel gene (CACNA1A). Neuropathology of SCA6 show predominant neuronal loss of the Purkinje cell in the cerebellum with formation of cytoplasmic aggregation of the a1A-calcium channel protein (Ishikawa, et al. Hum. Mol. Genet. 1999). However, the pathogenic mechanism by which mutated CACNA1A leads to cell death is poorly understood. To clarify the pathophysiology of SCA6, we analyzed the cell toxicity mediated by the expansion of the CAG repeat in CACNA1A in cultured cell system. The full-length CACNA1A with normal-length (13 CAG repeat unit) or expanded (28 repeat unit) repeats were fused with green fluorescent protein (GFP) cDNA, and were transiently expressed in human embryonic kidney (HEK)-293 cells and PC12 cells. While the fusion protein with normal-length polyglutamine was expressed diffusely in the cytoplasm, the fusion protein with expanded polyglutamine accumulated densely in the perinuclear cytoplasm in most occasions. In addition, a higher number of cells transfected with full-length CACNA1A with expanded CAG repeat underwent cell death with positive TUNEL staining, while cells transfected with CACNA1A with normal length CAG repeat appeared normal. Moreover, expanded polyglutamine in a portion of CACNA1A did not induce cell death. The present data provide evidence that the full-length a1A-calcium channel protein with expanded polyglutamine could be toxic to cells.

Program Nr: 2638 from the 1999 ASHG Annual Meeting

Screening and detection of variants in the Endothelin B Receptor Gene (EDNRB) : A candidate for asthma. *S.D. Pandit[1], S. Cheng[1], J. Dupuis[1], P. Van Eerdewegh[1,2], T. Keith[1].* 1) Human Genetics, Genome Therapeutics Corporation, Waltham, MA; 2) Department of Psychiatry, Harvard Medical School, Boston, MA.

Several studies have localized a putative candidate gene for asthma in the 13q14-q22 region. Among the known genes and ESTs that map in the candidate region, the endothelin B receptor gene (EDNRB) is particularly interesting because of its possible involvement in the pathophysiology of asthma. Endothelins (ET 1, 2 and 3) and their receptors, endothelin receptor type A and type B are highly expressed in human lung. Endothelins cause contraction of airway smooth muscle and this effect is predominantly mediated through the endothelin B receptor. Accordingly we analyzed the EDNRB for mutations using single stranded confirmation polymorphism (SSCP) analysis followed by sequencing of variants. The genomic structure of EDNRB gene has been elucidated previously (Arai, H., (1993), JBC, 268: 3463-3470). A total of ten primer pairs were utilized to cover the seven exons and an additional eight primer pairs were utilized to cover the promoter region. Eight out of the ten primer pairs covering the exons were redesigned to amplify smaller products (<250 bp) more amenable for SSCP analysis. Initially, we screened 40 unrelated asthmatics and 40 unrelated CEPH individuals for variants. Subsequently we identified and screened 51 unrelated asthmatic individuals from our collection of families that showed sharing by descent in affected for the 13q14-q22 chromosomal region. We have identified a total of eight variants in the EDNRB gene and confirmed the nucleotide changes by sequencing. Three of the variants (novel) were in the promoter region and five (2 novel) were in the coding region. Among the five variants detected in the coding region, three lead to amino acid substitutions of which one was novel. Statistical analyses were performed and no significant association of any to the asthma phenotype was observed (all p-values > 0.22). The current sample size could detect differences of 22% or more in allele frequencies between the asthmatic and non-asthmatic groups with 80% power using a two-sided test with type 1 error of 5%.

Program Nr: 2639 from the 1999 ASHG Annual Meeting

A common disease haplotype segregating in spinocerebellar ataxia 2 (SCA2) pedigrees of diverse ethnic origin.

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The autosomal dominant cerebellar ataxias (ADCAs) are a clinically heterogeneous group of neurodegenerative disorders. Genetic heterogeneity has been demonstrated and six of the disease genes have now been cloned - spinocerebellar ataxia 1 (SCA1), SCA2, Machado Joseph disease/SCA3, SCA6, SCA7 and SCA8. The identification of a CAG trinucleotide repeat expansion, located within the coding sequence of the ataxin-2 gene, as the mutation underlying SCA2 has facilitated direct investigation of pedigrees previously excluded from linkage analysis due to insufficient size or pedigree structure. With the advent of markers known to lie in close physical proximity to SCA2, it is now feasible to undertake a comparative study of the disease haplotypes segregating in pedigrees of diverse geographical and ethnic origin. We have previously described the identification of the ancestral disease haplotype segregating in the Cuban founder population used to assign the disease locus to chromosome 12q23-24.1. Using microsatellite loci within and flanking the SCA2 gene [D12S1328 - (0.03) - D12S1332 - (0.00) - D12S1672 - (0.00) - D12S1333 - (0.01) - D12S1329], haplotype analysis has been undertaken in key affected individuals from 7 Indian, 16 Cuban, one English and 3 Japanese families. Our results demonstrate the segregation of the identical core haplotype in 23 of these pedigrees at the loci D12S1672 and D12S1333 located 20kb proximal and 200kb distal to the triplet repeat motif, respectively. To establish the significance of association between the disease and alleles at the polymorphic loci segregating in these families, unrelated individuals derived from the respective ethnic populations were also genotyped. Interpretation of our data is suggestive that for these SCA2 pedigrees at least, the mutation has arisen on a single ancestral or predisposing chromosome.

Program Nr: 2640 from the 1999 ASHG Annual Meeting

Aging and frequency of replication/ transcription errors in poly(A)/(T) runs of human genes. *A. Paoloni-Giacobino[1], C. Rossier[1], V. Lambert[2], S.E. Antonarakis[1].* 1) Division of Medical Genetics, Geneva University Medical School, Geneva, Switzerland; 2) Department of Medecine, Geneva University Hospital, Switzerland.

The goal of this study was to estimate the error rate of the complex machinery of nuclear gene expression and its possible age-related increase. Mutant transcripts and therefore aberrant proteins may be associated with aging and errors could occur at nucleotide runs such as poly(A)s or poly(T)s. Toward this goal we compared the occurrence of polymerase slippage errors during replication and transcription in A or T runs in both DNA and RNA from young (18 y.o.) and old (80 y.o.) healthy individuals. We selected to analyze the (A)/(T) runs of 3 human genes: a stretch of 8 (A)s in TPRD, 10 (A)s in TGFBR2 (within the coding region of these two genes), and 13 (T)s in the 3'-UTR of ATRX. Total RNA and genomic DNA from blood were isolated from each individual. The error rate in DNA molecules was determined by sequencing 100 cloned PCR fragments containing the nucleotide run. The error rate in RNA molecules was determined by sequencing 100 cloned RT-PCR fragments (primers were choosen in different exons so that contamination with DNA amplification was avoided). The error rates observed in the different stretches were always higher in the RNA than in DNA for each of the genes studied. The error rate in replication and/or transcription was associated with the length of the stretches of poly(A)/(T) runs: e.g. the errors were 5%, 22% and 58% in the RNAs of the 8(A), 10(A) and 13(T) repeats, respectively, in the "old" samples. Moreover, the observed error rate was higher in old than in young individuals: e.g. in 13(T) the differences were 10% for DNA (28% vs 38%) and 5% in RNA (53% vs 58%). To exclude that the observed errors were due to Taq polymerase, we analysed 100 clones obtained from PCR on a plasmid containing the stretch of 13(T) and observed only 1 error. The preliminary conclusion from these data is that aberrant proteins are always produced due to the replication/transcription errors and that their rate of production increases with age.

Program Nr: 2641 from the 1999 ASHG Annual Meeting

Parkinson's disease and monoamine oxidases A and B: Association with MAO-B Alleles. *A. Parsian[1,2], B. Racette[2], Z.H. Zhang[1], M. Rundle[2], J.S. Perlmutter[2].* 1) Dept Psychiatry, Washington Univ Medical Sch, St Louis, MO; 2) Dept Neurology, Washington Univ Medical Sch, St Louis, MO.

Idiopathic Parkinson's disease (PD) is an age dependent, neurodegenerative disorder and is predominantly a sporadic disease. Only 20-30% of patients have a positive family history for PD with complex mode of inheritance. Monoamine oxidases A and B (MAO-A and B)are involved in the serotonin and dopamine metabolism. The neurotoxin MPTP requires activation by MAO-B to be toxic to dopaminergic cells and causes parkinsonian symptoms. The inhibition of MAO-B with drugs such as selegiline blocks MPTP induced neuronal degeneration in primates and reduces the rate of PD progression in humans. There have been reports of inconclusive association between PD and an A/G transition in intron 13 of the MAO-B gene (Kurth et al., 1993; Ho et al., 1995; Costa et al., 1997) in Caucasians. Recently, Mellick et al., (1999) reported a highly significant association between a large allele of the dinucleotide repeat in the intron 2 of MAO-B and PD ($p<0.00005$) in an Australian sample. To determine the role of MAO-A and B in the development of PD, we have screened a well characterized sample of 89 patients with, 168 patients without a positive family history (at least one first degree relative with definite PD), and 95 matched controls with dinucleotide repeat markers in both genes. Comparison of the PD groups with normal controls for the MAO-B allele frequencies was positive (PD with history $p=0.012$, PD without history $p=0.019$). The frequency of the large alleles (1 and 2) in PD group with a positive family history was highly significantly different than controls ($p=0.0033$). However, comparison of the PD groups with and without a positive family history to controls for the MAO-A gene polymorphism was negative ($p=0.089$ and $p=0.282$, respectively). Our data indicate that MAO-B may play a role in the susceptibility to familial form of the PD.

Program Nr: 2642 from the 1999 ASHG Annual Meeting

Exploring mutant huntingtin's conformational properties *in vivo*. *F. Persichetti[1], F. Trettel[1], V. Wheeler[1], C. Huang[1], S. Namura[2], L. Hirt[2], M. Timmers[3], M. Moskowitz[2], J. Gusella[1], M. MacDonald[1]*. 1) Molecular Neurogenetics Unit, Massachusetts General Hospital, Charlestown, MA; 2) Departments of Neurosurgery and Neurology, Massachusetts General Hospital, Charlestown, MA; 3) Laboratory for Physiological Chemistry, Utrecht University, Utrecht, The Netherlands.

Huntington's disease (HD) is caused by an expanded CAG repeat that lengthens a glutamine tract in huntingtin (htt)protein. A conformational change conferred by elongated glutamine may trigger the progressive neurological phenotype. Amino-terminal htt aggregates found in HD post-mortem brain may be causative in the disease, or may be the end products of a toxic process operating in the context of the full-length protein, possibly via interaction with normal or abnormal protein partners. We have examined the physical properties of mutant htt aggregates formed in cell culture from either truncated or full length proteins. Complexes derived from the truncated amino-terminal protein frequently present a conformational change that eliminate the reactivity with the glutamine specific mAb 1F8, coincident with the production of insoluble material. By contrast, complexes formed by the full-length protein retain their reactivity to 1F8 and do not generate insoluble aggregates. It is possible that the soluble full-length complexes foster non-covalent interactions with other proteins that could play an important role in HD pathogenesis. Interaction of an htt amino-terminal fragment with caspase *in vitro*has suggested glutamine-dependent cleavage, with apoptotic consequences. Therefore, we have also examined accurately expressed mutant htt in *Hdh*CAG knock-in mouse brain and have failed to find evidence of stable htt fragments or apoptosis. By contrast *Hdh*knock-in brain, in an *in vivo*apoptotic model (focal ischemia), accumulates a stable carboxy-terminal htt fragment, concomitant with apoptotic neuronal cell death. Differential cleavage of mutant and normal htt may suggest an altered conformational property of the mutant protein. Our inability to detect htt cleavage products in *Hdh*CAG knock-in brain suggests, therefore, that mutant htt triggers a subtle rather than an 'acute' pathogenic process, consistent with HD's late age at onset.

Program Nr: 2643 from the 1999 ASHG Annual Meeting

Analysis of the 4-1BB Gene in the NOD Mouse: A Positional Candidate for the *Idd9* Locus. *M.S. Phillips[1], P.A. Lyons[2], N. Armitage[2], P.J. Hey[1], L.S. Wicker[3], L.B. Peterson[4], M.L. Metzker[1], C.T. Caskey[1], J.A. Todd[2], J.W. Hess[1]*. 1) Dept. of Human Genetics, Merck Research Laboratories, West Point, PA; 2) Dept. of Medical Genetics, Cambridge Institute of Medical Research, WellcomeTrust/MRC Building, Addenbrokes's Hospital, Cambridge, UK; 3) Dept. of Autoimmue Disease Research, Merck Research Laboratories, Rahway, NJ; 4) Dept. of Pharmacology, Merck Research Laboratories, Rahway, NJ.

The nonobese diabetic (NOD) mouse is an animal model for human type 1 diabetes or autoimmune insulin-dependent diabetes mellitus. The NOD mouse also serves as a model for studying complex polygenic diseases. Currently, 16 loci that contribute to the development of type 1 diabetes in the NOD mouse have been mapped by linkage analysis. To fine map these loci, we used the strategy of congenic mapping by breeding a diabetes resistant mouse strain, C57BL/10, onto an NOD background. Using this approach, we localized the *Idd9* locus to a 2.1-cM interval on chromosome 4 containing several members of the TNF receptor superfamily, including the 4-1BB gene (CD137 or TNFRSF9).

The 4-1BB gene represents a good positional candidate for the *Idd9* locus since it is involved in the activation of T-lymphocytes. Using both cDNA and genomic sequencing, the 4-1BB gene was analyzed in six different mouse strains to determine polymorphic differences. Several polymorphic changes between NOD and B10 were identified, including two amino acid changes.

Program Nr: 2644 from the 1999 ASHG Annual Meeting

Hypomethylation of an expanded *FMR1* allele is not associated with a global DNA methylation defect. *B.W. Popovich[1], R.W. Burman[1], P.A. Yates[2], L.D. Green[1], P.B. Jacky[3], M.S. Turker[1,2]*. 1) Dept of Molecular and Medical Genetics, Oregon Health Sciences University, Portland, OR; 2) Center for Research on Occupational and Environmental Toxicology, Oregon Health Sciences University, Portland, OR; 3) Cytogenetics Laboratory, Kaiser Permanente NW, Clackamas, OR.

The fragile X syndrome is a mental retardation disorder caused by expansion of a CGG trinucleotide repeat in the 5' UTR of the *FMR1* gene. The vast majority of full mutations are heavily methylated throughout the expanded CGG repeat and the surrounding CpG island. Hypermethylation of promoter elements is associated with transcriptional silencing of the *FMR1* gene. The resulting decrease or absence of FMR1 protein (FMRP) is believed sufficient to cause the fragile X syndrome phenotype. The relation between repeat expansion and hypermethyaltion is not well understood, nor is it absolute as demonstrated by the identification of non-retarded males who carry full mutations that lack methylation at some sites in the promoter and produce some FMRP. To better characterize the methylation pattern in a hypomethylated full mutation carrier, we have evaluated methylation at numerous sites in the *FMR1* CpG island including those located within the CGG repeat. We find that the expanded CGG repeat and 1 kb or surrounding DNA are free of methylation in this full mutation male.

Methylation of the CGG repeats has been hypothesized to occur when the repeat is expanded sufficiently to resemble a potentially transposable repetitive element. To better understand the basis of the *FMR1* methylation deficiency in the full mutation male, we have assessed methylation levels at selected linked and unlinked repetitive element loci. We find normal methylation levels at *Alu* elements flanking the CGG repeat and at other repetitive element loci. This result demonstrates that the lack of methylation in the CGG repeat region is not associated with a global defect in methylation of highly reiterated DNA sequences. We also report that *de novo* methylation of the expanded CGG repeat region does not occur when it is moved via microcell mediated chromosome transfer into a *de novo* methylation competent mouse cell line.

Program Nr: 2645 from the 1999 ASHG Annual Meeting

Identification of a premature ovarian failure candidate gene. *R.L. Prueitt[1], J.L. Ross[2], A.R. Zinn[1].* 1) McDermott Center & Department of Internal Medicine, UT Southwestern Medical School, Dallas, TX; 2) Department of Pediatrics, Thomas Jefferson Univ, Philadelphia, PA.

Premature ovarian failure (POF) is defined as cessation of menses (amenorrhea) associated with elevated serum gonadotropins occurring in women before the age of 40. Roles for the environment, autoimmunity, and X chromosome abnormalities in the etiology of POF have been described. A region of the X chromosome from Xq13 to Xq26 is critical for normal ovarian development. Women with balanced translocations involving this region of the X chromosome often exhibit POF. Currently, neither the basis for the large critical region nor the mechanism(s) by which these translocations cause POF is known. It has been proposed that many different genes that are essential for normal ovarian function are present in this region.

We have collected cell lines from 14 patients with balanced Xq;autosome translocations. Nine of these translocations were associated with POF. To date, we have mapped eight Xq breakpoints to the limits of resolution of the X chromosome physical map using somatic cell hybrids. One breakpoint within Xq25 in a subject with 2° amenorrhea interrupts the gene encoding aminopeptidase P, *XPNPEP2*. Aminopeptidase P hydrolyzes N-terminal X-Pro bonds that are present in various hormones, growth factors, and cytokines. Soluble and insoluble forms of the protein have been isolated from brain, serum, platelets, leukocytes, kidney, and lung. So far the only known biological substrate is the vasodilator bradykinin, but because of the nature of its other potential substates, aminopeptidase P may have a hormone-processing or immunologic function which could define its role in POF.

Another candidate POF gene within the critical region, *diaphanous* has been previously reported to be disrupted by a balanced translocation in a woman with 2° amenorrhea. We conclude that *XPNPEP2* is also a candidate POF gene, supporting the theory that the critical region contains multiple genes required for normal ovarian development and function.

Program Nr: 2646 from the 1999 ASHG Annual Meeting

Mechanisms of CTG-repeat induced changes in gene expression. *G. Raca, E. Siyanova, S. Mirkin.* University of Illinois, Chicago.

Myotonic Dystrophy is the most common neuromuscular disease affecting adults. The molecular change responsible for the disease is an expansion of the normally polymorphic CTG repeat in the 3' UTR of the DM protein kinase gene. It is not yet known how the amplified repeat induces the complex and polymorphic phenotype of Myotonic Dystrophy. At least three different mechanisms were proposed: a) altered expression of the gene containing the repeat (DMPK) b) decreased expression of the downstream gene (DMAHP/SIX5) and c) effects mediated at the RNA level, possibly caused by the CUG-containing RNA structure. To explore the mechanisms through which long CTG repeats can interfere with gene function, we cloned CTG/CAG repeats of different lengths at various positions relative to the luciferase gene and analysed their effects on reporter gene expression upon transient transfection into the human colon carcinoma SW480 cells. We found that these repeats caused a significant decrease in luciferase activity only when positioned in the 5'UTR of the gene. The effects were much stronger with CTG in the transcribed strand (orientation dependence) and for longer repeats (length dependence). RNase protection studies showed no difference between the control and different constructs in the amount of luciferase RNA, demonstrating that a transcription block was not responsible for the observed decrease in gene activity. However, in vitro translation using reticulocite lysates demonstrated that CTG repeats located in the 5' UTR reduce protein levels relative to the control. In fact, the same type of orientation and length dependence was observed with CTG repeats in both the in vivo expression assay and in the in vitro translation experiments. We suggest that the formation of RNA hairpins by CUG repeats can explain our data. Such hairpins could block scanning through the 5' UTR by a small ribosomal subunit. While the formation of RNA hairpins by CUG repeats in vitro has been described before, the results of our expression studies suggest, for the first time, that such structures can also exist in vivo.

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Program Nr: 2647 from the 1999 ASHG Annual Meeting

MULTIPLE SCLEROSIS AND RETINITIS PIGMENTOSA SEGREGATE IN A LARGE WELSH KINDRED.

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Multiple Sclerosis (MS) is the most common neurological disorder among young adults and affects around 85,000 people in UK. This is an autoimmune disorder which usually causes demyelination in genetically susceptible individuals. By genome wide screening several groups found that MS has association with several chromosomes (1p, 6p/q, 7p, 14q, 17p/q, 19q, x cen). The strongest association was found with the Major Histocompatibility Complex (MHC Ch6p21.3) especially HLA class II, DR15 haplotype. In our ongoing study the association of multiple sclerosis with the MHC, we are studying a family from South Wales where MS and Retinitis Pigmentosa (RP) were found to be segregated in four generations. Retinitis Pigmentosa (RP) is a progressive hereditary disorder of the retina of which various forms of have already been described. An autosomal dominant form of RP has been localised to Ch17q11.2 and an X linked RP (RPLX) was found at Ch Xp21.2 locus. In this kindred a male patient age, 45 suffered from a progressive type of MS, his mother also suffer from reminent-relapsing (RR) type MS and RP, grandmother was blind due to RP, maternal cousin and his Nephew suffer from progressive type of MS. The mode of inheritence of these disorders in this family has yet to be elucidated, but appears to be either autosomal dominant or X linked. We have found association with the myelin protein on Ch6, together with an association at Ch17q11.

Program Nr: 2648 from the 1999 ASHG Annual Meeting

Massive CTG expansions and deletions may reduce penetrance of spinocerebellar ataxia type 8. *L.P.W. Ranum[1], M.L. Moseley[1], M.F. Leppert[2], G. van den Engh[3], A.R. La Spada[3], M.D. Koob[1], J.W. Day[1].* 1) Genetics Cell Biol & Devel, Inst Hum Genet and Neurology, Univ Minnesota, Minneapolis, MN; 2) Hum Genet, Univ Utah, Salt Lake City, UT; 3) Univ Wash Med Ctr, Seattle, WA.

We recently isolated an untranslated CTG expansion that causes a novel ataxia (SCA8). Unlike other triplet diseases, SCA8 may have a window of pathogenic alleles (~110-250 repeats) with shorter and larger repeat tracts not resulting in disease. In our largest family (z=6.8), affecteds have 107-127 CTG repeats while repeats <100 are not associated with disease. Among 11 families, affecteds have up to 250 repeats. Although expanded alleles are transmitted from males or females, 26/30 affecteds inherited maternally transmitted expansions. This maternal penetrance bias is consistent with paternal contractions resulting in alleles shorter than the pathogenic threshold, and maternal expansions yielding alleles within the pathogenic range. In some cases, maternal transmissions result in very large repeats (250-800 CTGs). In two families, unaffected men (73 and 79 yrs) with 260 and 300 CTG repeats transmitted shorter pathogenic alleles to four affected offspring. In addition, out of 1200 control chromosomes, one allele of 800 repeats was found in a CEPH grandmother. Medical histories indicate that neither this woman nor her son (54 yrs, 800 repeats), are affected by ataxia. These observations indicate that very large alleles (>250 repeats) may not be pathogenic, possibly because they are not expressed or because of altered RNA processing or stability. In this CEPH family, four children inherited the expanded allele from their father, but in each case, the alleles underwent massive deletions (~700 repeats) resulting in 85, 93, 116 and 118 repeats. To further investigate the instability of very large alleles, we performed Southern analysis on sperm DNA from men with 500 and 800 repeats. In both cases, nearly all of the expanded alleles underwent massive deletions into narrow size ranges (~80 and ~110 CTGs) typically at or below the pathogenic threshold. Massive paternal deletions and maternal expansions may minimize moderately sized pathogenic alleles and play a role in the reduced penetrance of SCA8.

Program Nr: 2649 from the 1999 ASHG Annual Meeting

A new Digestion Pattern in Patients with Myoclonic Epilepsy and Ragged-Red Fiber Disease (MERRF) depending on the Ethnic Origin of the Individuals. *C. Rodas[1], J.C. Prieto[1], G. Keyeux[1], E. Espinosa[2], J.E. Bernal[1], A. Rotig[3], A. Munnich[3].* 1) Inst Genetica Humana, Univ Javeriana, Bogota, DC, Colombia; 2) Hospital Militar Central, Bogota; 3) U Genet Enfants Malades, Hopital Necker, Paris.

Myoclonic Epilepsy and Ragged-Red Fiber Disease, MERRF, is a maternally inherited disease characterized by progressive myoclonic epilepsy, a mitochondrial myopathy with RRFs, and a slowly progressive dementia. Approximately 80 to 90 percent of MERRF cases are caused by a heteroplasmic G to A point mutation at nt 8344. Additional cases have been attributed to a heteroplasmic T to C transition mutation at nt 8356 in the tRNALys gene. We analyzed mtDNA from 10 healthy control and 22 patients with probable MERRF disease for the mutations 8344 and 8356, using mutation-specific restriction endonuclease digestions (BglI and XbaI). Results: For position 8344: in 20 samples we obtained a band corresponding to the expected 187bp size, in 11 samples a band of 178bp and in one sample a band of 205bp. For position 8356: in one affected patient we obtained a three-band pattern, corresponding to the expected 88bp, 69 bp and 27bp cleavage products of the XbaI constitutive and mutation sites; 19 samples with two bands of 96bp and 88bp corresponding to the normal expected fragments without mutation; 11 samples with two bands of 96bp and 79bp, and one subjects with two bands of 106bp and 96bp. A 9-bp deletion between the COII and tRNALys genes, due to the lack of one of the two adjacent copies of a 9-bp sequence (5`CCCCCTCTA 3), has been described in some populations. The PCR primers used in our study for screening mutations 8344 and 8356 amplify sequences from the COII gene, the intergenic region and the tRNA Lys gene. We therefore reamplified the DNAs yielding unusual restriction patterns with the 9bp-region specific primers, and show that the PCR generates fragments with different sizes depending on the number of copies of the 9bp-sequence. Thus, in the analysis of the mutations in patients from different ethnic origins with MERRF, it is important to considerate the polymorphism in the intergenic region which may lead to erroneous interpretations.

Program Nr: 2650 from the 1999 ASHG Annual Meeting

The Turner syndrome-associated neurocognitive phenotype maps to distal Xp. *J.L. Ross[1], H. Kushner[2], A. Zinn[3].* 1) Pediatrics, Thomas Jefferson Univ, Philadelphia, PA; 2) Biomedical Computer Research Institute, Phila., PA; 3) University of Texas Southwestern Medical School, Dallas, TX.

Turner syndrome (monosomy X, TS) is associated with a characteristic neurocognitive profile including impaired visual-spatial/perceptual abilities in children and adults. We propose that this visual-spatial/perceptual deficit is genetically determined on the basis of haploinsufficiency of specific X chromosome gene/genes that escape X inactivation. We utilized a molecular approach and genotype/phenotype correlations to identify critical regions of the X chromosome for neurocognitive aspects of TS. The study population included 33 females with partial monosomy for Xp (ages 7-40 years). Deletions were mapped by FISH with a panel of Xp markers and, in some cases, by loss of heterozygosity of polymorphic markers. We previously identified, through discriminant function analysis, a composite formula for the TS-associated neurocognitive phenotype that optimally distinguished TS children and adults from female age-, Verbal IQ-, and SES-matched controls (Z-scores from 6 visual-spatial/perceptual tasks). Statistical criteria with optimal sensitivity and specificity were chosen for labeling an individual as having the TS-associated neurocognitive phenotype, and 11/33 partial deletion subjects met the specific phenotype criteria. Seven of these 33 subjects had terminal Xp delations distal to Xp22.3. Their mean spatial cognitive score was significantly lower than that of 448 controls (-5.9 +/- 3.9 versus 0.2 +/- 4.9, $P<0.001$) and was similar to the mean score of 208 TS children and adults (-7.1 +/- 4.9). All 7 terminal Xp deletion subjects has scores below the mean for the normal controls ($P<0.02$). One other subject with an interstitial deletion of Xp22.13-Xp22.32, who was not missing all of Xp22.33, had a normal spatial cognitive score (=0.03). In summary, females missing only the distal third of the short arm of the X chromosome manifested the characteristic TS-associated neurocognitive profile. We conclude that the TS neurocognitive phenoype maps to mid- or distal Xp22.3 in children and adults with partial monosomy Xp. Future studies will define the interval more precisely.

Program Nr: 2651 from the 1999 ASHG Annual Meeting

Molecular Characterization of Multiple Mitochondrial DNA Deletions of Ten Individuals. *C.A. Rupar[1,3,4], C. Regan[1,2]*. 1) Biochemical Genetics Laboratory, CPRI; 2) Dept Zoology; 3) Dept Biochemistry; 4) Dept Paediatrics, University of Western Ontario, London, Canada.

Large-scale deletions of the human mitochondrial genome are linked to a number of neuromuscular disorders associated with deficiencies of cellular energy production. Single site deletions are most often associated with classical mitochondrial myopathies such as Kearns-Sayre syndrome, whereas multiple site deletions are associated with an increasing variety of disorders and occur as part of the normal aging process. Multiple deletions were characterized in the mitochondrial genomes of ten individuals, aged twenty-two to seventy-seven, with a variety of neuromuscular symptoms. Most individuals presented with at least one of ptosis, proximal limb weakness or progressive external ophthalmoplegia and one or more additional neurological symptoms. Long-distance PCR, across a 10.4 kb region in which most mitochondrial deletions have been reported to occur, yielded multiple shorter amplicons from muscle DNA. Amplification using leukocyte DNA available from three individuals yielded only the normal 10.4 kb fragment. Fragments amplified across deletions were cloned and sequenced to determine deletion breakpoints. In thirty-three of fifty deletions detected in the ten individuals the 3' breakpoint was observed between nucleotide 16047 and nucleotide 16075. This lies in the control region of human mitochondrial DNA and indicates a potential protected region of the mitochondrial genome. Both direct repeats of 5-10 bp and small overlaps of 1-8 bp were frequently observed around breakpoints. In thirty-one of fifty deletions at least one of the direct repeats occurred within 10 bp of the breakpoint. Overlaps were seen in twenty-two of fifty deletions. These features are consistent with the emerging evidence that recombination and repair mechanisms are responsible for the generation of mitochondrial DNA deletions.

Program Nr: 2652 from the 1999 ASHG Annual Meeting

Reactivation of imprinted genes in Prader-Willi syndrome. *S. Saitoh, T. Wada.* Dept. Pediatrics, Hokkaido Univ., Sapporo, Japan.

Genomic imprinting results from a parent-of-origin specific gene modification, with monoallelic expression of imprinted genes. DNA methylation is associated with this inactivation process. However, the mechanism of imprinted gene inactivation is not fully understood. Chromosome 15q11-q13 harbors several imprinted loci involved in Prader-Willi syndrome (PWS). *SNURF-SNRPN* is an imprinted locus in 15q11-q13, and the imprinted status is stably maintained in somatic tissues and cell lines. The promoter region of *SNURF-SNRPN* is completely methylated on the inactivated maternally-derived allele, and unmethylated on the active paternally-derived allele. *In vitro* experiments showing that methylation eliminates promoter activity suggest methylation plays an important role in inactivation of *SNURF-SNRPN* . To better understand the role of methylation in regulation of imprinted genes, we treated lymphoblastoid cell lines from five PWS patients with a paternal deletion or maternal uniparental disomy, thus carrying only maternal alleles of 15q11-q13, with a demethylating agent, 5-aza-dC. Expression of two imprinted genes in 15q11-q13, *SNURF-SNRPN* and *IPW*, which were normally inactivated, was demonstrated by RT-PCR after 5-aza-dC treatment. This expression was shown to be associated with demethylation of the CpG island of *SNURF-SNRPN* . These results indicate that suppression of some imprinted genes in 15q11-q13 is maintained by DNA methylation.

Furthermore, gene inactivation by DNA methylation has recently been demonstrated to be carried out through interaction with a deacetylation complex. We treated PWS cell lines with trichostatin A (TSA), an inhibitor of deacetylation. After TSA treatment, expression of *SNURF-SNRPN* and *IPW* was demonstrated by RT-PCR. However, the CpG island of *SNURF-SNRPN* remained hypermethylated based on data using methylation-sensitive restriction exzymes. These results further support the idea that DNA methylation-mediated inactivation of imprinted genes requires recruitment of a deacetylation complex. The findings also have possible implications for the potential of pharmaceutical treatment of imprinting related-disorders.

Program Nr: 2653 from the 1999 ASHG Annual Meeting

Rat Models of Autoimmune Diseases: The Genetic Dissection of Complex Traits. *J. Salstrom[1], T. Furuya[1], G.W. Cannon[2], E.F. Remmers[1], M.M. Griffiths[2], R.L. Wilder[1].* 1) Arthritis & Rheumatism Branch, NIAMS / NIH, Bethesda, MD; 2) Research Service Veterans Affairs Medical Center and Department of Medicine/Rheumatology, University of Utah, Salt Lake City, Utah.

Because genetic heterogeneity, phenotypic heterogeneity, and environmental noise complicate the study of complex traits in humans, inbred rat strains in controlled laboratory environments are being used extensively to aid in the dissection of many multifactorial traits such as autoimmune diseases. The BB(DR) rat is a particularly good model for autoimmune diseases because, although it is resistant to spontaneous autoimmune insulin-dependent diabetes mellitus (Iddm), many autoimmune diseases such as Iddm, thyroiditis, and collagen-induced arthritis (CIA), a model of rheumatoid arthritis (RA), can be induced by environmental perturbation. To identify loci that regulate autoimmune diseases, we crossed the BB(DR) rat with the CIA resistant BN/SsN (BN) rat and conducted a genome-wide scan for quantitative trait loci (QTL) that regulated CIA severity and autoantibody production in the F2 population. We then compared the locations of these loci with the locations of loci known to regulate other autoimmune diseases. Seven genomic regions were found to regulate arthritis severity, and three were found to regulate autoantibody production. Several of these overlapped with homologous loci that regulate experimental autoimmune encephalomyelitis (EAE) in rats, insulin-dependent diabetes (Idd) in mice, as well as RA and multiple sclerosis (MS) in humans. However, only two severity QTL and two autoantibody QTL overlapped with the seven QTL (five severity and two autoantibody) found to regulate arthritis severity and autoantibody production in a similar analysis of DA (highly susceptible to CIA) X BN F2 progeny. These data support the view that polygenic autoimmune diseases share numerous genetic risk factors, but also illustrate that many unique profiles of regulatory loci can be identified for a single disease. Study of autoimmune diseases in inbred rat strains provides a powerful complementary approach to the dissection of related complex traits in outbred populations.

Program Nr: 2654 from the 1999 ASHG Annual Meeting

Increased Apoptosis of Huntingtons Disease Lymphoblasts Associated with Repeat Length-Dependent Mitochondrial Depolarization. *A. Sawa[1], G.W. Wiegand[2], J. Cooper[3], R.L. Margolis[3], A.H. Sharp[3], J.T. Greenamyre[4], C.A. Ross[1,3], S.H. Snyder[1,3].* 1) Dept Neuroscience, Johns Hopkins Univ, Baltimore, MD; 2) National Cancer Institute, Frederick, MD; 3) Department of Psychiatry, Johns Hopkins Univ, Baltimore, MD; 4) Department of Neurology, Emory Univ, Atlanta, GA.

Huntingtons disease (HD) is a genetically dominant condition caused by expanded CAG repeats coding for glutamine in the HD gene product huntingtin (htt). Though HD symptoms reflect preferential neuronal death in specific brain regions, htt is expressed in almost all tissues of the body, so that one might anticipate abnormalities outside the brain. We now report that lymphoblasts derived from HD patients display increased stress-induced apoptotic cell death. HD lymphoblasts also manifest a marked increase in mitochondrial depolarization correlated with increased glutamine repeats. Apoptotic cell death is often associated with activation of caspase-3. Following STS treatment, caspase-3 activity is markedly greater in HD cells compared with control subjects. Caspase-9 is also more activated in HD, however, no difference is found in caspase-8 after Fas addition between HD and controls. These results suggest that specific subsets of caspases are activated by abnormal htt. We tested drugs for effects on the increased apoptotic death of HD cells. CsA and z-VAD-fmk significantly reduce STS elicited cell death. To assess specificity, we also examined lymphoblasts of SCA-1 patients, which do not differ from controls regarding caspase-3 activation and mitochondrial depolarization.

Program Nr: 2655 from the 1999 ASHG Annual Meeting

A genome-wide scan for quantitative trait loci for lipid levels: The Framingham Study. *A.M. Shearman[1], J.M. Ordovas[2], L.A. Cupples[3], E.J. Schaefer[2], M.D. Harmon[3], O. Joost[3], A.L. DeStefano[3], D. Keen[1], P.W.F. Wilson[4], D.E. Housman[1], R.H. Myers[3].* 1) Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139; 2) Lipid Metabolism Laboratory, USDA Human Nutrition Research Center on Ageing at Tufts University, Boston, MA 02111; 3) Boston University School of Public Health and School of Medicine, Boston, Massachusetts 02118; 4) National Heart, Lung, and Blood Institute's Framingham Heart Study, Framingham, MA 01701.

There is a significant association between cardiovascular disease and plasma lipid and lipoprotein levels. These traits show high genetic heritabilities as well as significant environmental effects. A genome-wide scan for plasma total cholesterol (TC) level, triglyceride (TG) level, triglyceride/HDL-cholesterol (TG/HDL-C) ratio and total cholesterol/HDL-cholesterol (TC/HDL-C) ratio was carried out in 237 families of the Framingham Study. The families included 1167 individuals that were genotyped. Multipoint lod scores were calculated for the four quantitative traits, using the variance component methods of the SOLAR program. There were 21 regions with multipoint lod scores greater than 1, on chromosomes 1, 3, 4, 5 (2 peaks), 7, 8, 11, 13 (3 peaks), 14, 15, 16, 17, 18, 20, 21. The maximum multipoint lod score for TC was 1.9 on chromosome 8 (at 100 cM), for logTG was 1.8 on chromosome 14 (at 126 cM), for TC/HDL-C was 2.4 on chromosome 13 (at 91 cM) and for TG/HDL-C the maximum lod scores were 2.0 on chromosome 7 (at 147 cM) and 2.8 on chromosome 20 (at 48 cM). These results suggest regions worthy of further exploration.

Program Nr: 2656 from the 1999 ASHG Annual Meeting

Lack of age-associated LFA-1 upregulation in lymphocyte from Down syndrome patients. *C.C. Shieh, R.F. Lee, S.J. Lin.* Pediatrics and Immunology, National Cheng Kung University, Tainan, Tainan, Taiwan.

Immunodeficiency in Down syndrome patients contributes to their susceptibility to infections. Excessive lymphocyte adhesiveness caused by overexpression of lymphocyte adhesion molecule-1 (LFA-1, CD11a CD18), of which the beta chain (CD18) is encoded in chromosome 21, was believed to cause abnormal immune development and responses. In order to understand the relationship between LFA-1 expression and immune development in DS, we analyzed the LFA-1 expression in lymphocytes from DS with CD11a and CD18 cell surface staining and flow cytometry. DS patients less than two years of age expressed higher level of LFA-1 when compared with age-matched controls ((MFI,mean±SEM) 242.1±36.4 vs. 128±20.6, $p<0.05$). The differences in LFA-1 expression were much less significant in older DS patients when compared with age matched children (2-6 year-old (yo) group: 205.8±15.2 vs. 194.8±40.5; 6-15 yo group: 265.8±33.0 vs. 287.5±89.9). Although older children (2-6 and 6-15 yo groups) without DS tend to increase their expression of lymphocyte LFA-1 when compared with young children (0-2 yo) ($p<0.05$), DS patients showed no age associated increase in lymphocyte LFA-1 expression. We further investigated whether abnormality in CD4 and CD8 subpopulations accounts for this LFA-1 expression pattern. Two-color analysis with CD4/CD8 and LFA-1 in patients over 2 yo showed that percentages of CD4+ lymphocytes were comparable in DS patients and controls, while percentages of CD8+ lymphocytes were higher in DS patients (39.3±3.2 vs. 29.2±3.2, $p<0.05$). Expression of LFA-1 on both CD4+ and CD8+ lymphocytes were higher in DS patients (CD4+: 1070±95.1 vs. 869.4±59.8; CD8+: 1936±217.5 vs. 1272±195.7). The observations that DS patients had higher percentage of CD8+ lymphocytes and that CD8+ cells tended to have higher expression of LFA-1 integrin suggest that change in CD4/CD8 ratio is not among the explanations that DS patients fail to upregulate their LFA-1 expression with ages. A generalized pathological process like early senescence of the immune system or incapability in converting to memory phenotye of lymphocytes may underlie this unique expression pattern of LFA-1 integrin in DS patients.

Program Nr: 2657 from the 1999 ASHG Annual Meeting

Molecular analysis of the untranslated $(CTG)_n$ trinucleotide repeat that causes SCA8. *I. Silveira[1], I. Alonso[1], P. Mendonça[1], M. Costa[2], L. Jardim[3], C. Barbot[1], J. Barros[4], P. Coutinho[5], J. Sequeiros[1]*. 1) UnIGENe, IBMC- Univ. Porto, Porto, Portugal; 2) Serv. Neurologia, Hosp. Pedro Hispano, Matosinhos; 3) Serv. Genética Médica, Hosp. Clínicas Porto Alegre, Brazil; 4) Serv. Neurologia, HGSA, Porto, Portugal; 5) Serv. Neurologia, Hosp. S.Sebastião, Feira, Portugal.

The autosomal dominant spinocerebellar ataxias (SCAs) are a group of late onset neurodegenerative disorders for which 10 loci have been mapped (SCA1,2,MJD,4,5,6,7,8,10 and DRPLA). The pathological proteins show an expanded polyglutamine tract in SCA1, SCA2, MJD, SCA6, SCA7, and DRPLA; a glycine-to-arginine substitution has also been shown in SCA6. Recently, an untranslated composite $(CTA)_n(CTG)_n$ expansion on chromosome 13q was found to cause a novel form named SCA8. In order to identify the molecular basis of neurodegeneration in our ataxia patients, we have assessed the size of this $(CTG)_n$ in our ataxia families. We studied 78 unrelated individuals belonging to 26 dominant families, nine apparently recessive kindreds and 43 isolated cases previously excluded for SCA1, SCA2, MJD/SCA3, SCA6, SCA7 and DRPLA; 63 families were of Portuguese origin, while 15 kindreds where from Brazil. Molecular analysis was performed by PCR amplification of the CTG repeat, and allele sizes were estimated by comparing migration relative to an M13 sequencing ladder. Two affected individuals from two families (3%) had an allele in the expanded range (over 107 CTA/CTG repeats); three other patients, representing three different families (4%) presented potentially pathogenic alleles (97 to 105 CTA/CTG repeats). The size of the normal alleles ranged from 18 to 36 CTA/CTG units. In one family the (unaffected) father, who had alleles with 82 and 170 repeats, transmitted an allele with 105 CTA/CTG units to his daughter, who has congenital ataxia. We are now sequencing expanded alleles, and determining the allele sizes of a control Portuguese population (2000 chromosomes). The SCA8 CTA/CTG repeat expansion may thus be responsible for a significant number of our SCA families.

Program Nr: 2658 from the 1999 ASHG Annual Meeting

Molecular analysis of a large panel of Dutch ataxia patients. *R.J. Sinke, C.M. Diepstraten, P.F. Ippel, J.K. Ploos van Amstel.* Dept Medical Genetics, Univ Medical Center Utrecht, Utrecht, Netherlands.

The hereditary ataxias are a heterogeneous group of progressive neurodegenerative disorders of which a reliable clinical classification is hampered by the great overlap of phenotypic features and the great variability found both within and between families. Also, the inheritance pattern is not always clear and a great genetic heterogeneity is observed. To date nine different loci have been identified in the etiology of autosomal dominantly inherited spinocerebellar ataxias (SCA) and one locus responsible for the autosomal recessively inherited Friedreichs ataxia (FRDA). In the corresponding genes the causative mutations are almost exclusively trinucleotide repeat expansions. For the SCA6 gene also point mutations have occasionally been detected in ataxia families. Here, we present our six years experience regarding the molecular analysis of the trinucleotide repeat expansions in SCA and FRDA genes. This provides insight in the incidence and relative frequency of the different mutations in our patient group. Our present panel consists of 388 Dutch families with familial or isolated ataxias. It comprises true autosomal dominant cerebellar ataxia families, families with unexplained, often sporadic, ataxias and Friedreichs ataxia families. Sequential analysis of the different trinucleotide repeats, viz.CAG (SCA1,2,3,6,and 7 genes), GAA (FRDA) and CTG (SCA8 gene), allowed the detection of SCA1 mutations in 2 %, SCA2 mutations in 2 %, SCA3 mutations in 9 %, SCA6 mutations in 7 %, SCA7 mutations in 4 %, and FRDA mutations in 4 % of all probands. The SCA8 locus is currently under investigation. In conclusion, we were able to identify the genetic defect in 28 % of the probands, with the SCA3 and SCA6 mutations being the most frequent ones in the Dutch population.

Program Nr: 2659 from the 1999 ASHG Annual Meeting

Twin Zygosity and Schizophrenia. *C.L. Smith[1], G. Nguyen[1], J. Bouchard[1], C. Foulon[1], L. Keith[2].* 1) Ctr Advanced Biotechnology, Depts. of Biomedical Eng, Biology and Pharmacology, Boston Univ, Boston, MA; 2) Dept. Ob. and Gyn., and Center for Study of Multiple Births, Northwester Univeristy.

Targeted genomic differential display was used to quantitate the level of genome similarity in twins presented to us as monozygotic and either discordant or concordant for Schizophrenia. Although this was a small sample size (10 pairs), two and possible three groups could be distinguished at the DNA level. One group has a DNA similarity level similar to that seen in sibling pairs, suggesting that this group was dizygotic rather than monozygotic. At the other extreme was a group that had a DNA similarity level greater than that of sibling pairs, suggesting that this group was monozygotic. An intermediate group was also distinguished. The intermediate group were, possibly, blood stem cell chimerics. All of the twin pairs concordant for Schizophrenia were in the group we classified as monozygotic whereas all the twins discordant for Schizophrenia were in the group classified as dizygotic. In contrast to earlier studies, the results suggests that Schizophrenia is clearly a predominantly genetic disease with high penetrance. These results bring into questions a large body of research of that depended on accurate twin zygosity determinations for measuring heritability and penetrance of disease.

Program Nr: 2660 from the 1999 ASHG Annual Meeting

Mutation analysis of myelin oligodendrocyte glycoprotein in dyslexic siblings. *S.D. Smith[1], P.M. Kelley[2], J. Askew[2].* 1) Center for Human Molecular Genetics, Munroe Meyer Institute, University of Nebraska Medical Center, Omaha, NE; 2) Boys Town National Research Hospital, Omaha, NE.

Linkage analyses by our group and two others have localized a gene influencing specific reading disability (dyslexia) to an approximately 2 cM region of 6p21.3 (Gayan et al., 1999; Fisher et al., 1999; Grigorenko et al., 1997) . The myelin oligodendrocyte glycoprotein (MOG; MIM#159465) gene which maps to this region was selected as a candidate. MOG is a membrane protein, a member of the immunoglobin superfamily, that is found on the outermost lamellae of mature myelin. Although the exact function of this gene is unknown, its presence in the CNS and the hypothesized relationship between dyslexia and temporal processing rate (Livingtson et al, 1991), as well as a suggested relationship with intelligence by association analysis (Plomin et al., 1994) and a controversial relationship between dyslexia and immune disorders, made it a candidate for dyslexia. Analysis of the coding exons and adjacent splice sites in a subset of 22 dyslexic children from 10 sibships found a missense mutation in exon 3 in two of the sibships. This change from the published sequence occurred in 86 of 96 random controls, making it considerably less frequent in this small sample of dyslexics. We are examining the segregation of this SNP in our overall population of 150 sib pairs to determine if it is in disequilibrium with the reading disability locus.

Program Nr: 2661 from the 1999 ASHG Annual Meeting

Targeted genome screen of panic disorder and anxiety proneness using homology to murine QTL regions. *J.W. Smoller[1], J.S. Acierno[2], J.F. Rosenbaum[1], M.H. Pollack[1], J. Biederman[1], C. White[1], L. Helbling[2], J.F. Gusella[2], S.A. Slaugenhaupt[2]*. 1) Dept Psychiatry,; 2) Molecular Neurogenetics Unit, Massachusetts General Hosp, Boston, MA.

Family and twin studies have indicated that genes influence the susceptibility to panic and phobic anxiety disorders but the location of the genes involved remains unknown. Animal models can simplify gene mapping efforts by overcoming problems that complicate human pedigree studies including genetic heterogeneity and high phenocopy rates. Synteny homology between rodent and human genomes can be exploited to map human genes underlying complex traits. We used regions identified from QTL-mapping of anxiety phenotypes in mice to guide a linkage analysis of a large multiplex pedigree (103 members, 73 genotyped) segregating panic disorder/agoraphobia. Two phenotypes were studied: panic disorder/agoraphobia and a phenotype ("D-type") designed to capture early-onset susceptibility to anxiety disorders. A total of 103 markers across 10 chromosomal regions were typed. Parametric lod acore analysis was performed using parameters derived from a segregation analysis of panic disorder. In simulations, the maximum expected lod scores = 4.68 for panic disorder and 6.04 for D-type. One lod score > 2.0 was detected: lod = 2.4 at a locus on chr 10 under a reduced penetrance dominant model for the anxiety-proneness (D-type) phenotype. This finding was not supported in a nonparametric NPL analysis. Nonparametric analysis revealed interesting regions on chr 12 for panic disorder (NPL = 3.82, p =.009) and chr 1 for D-type (NPL = 1.99, p =.04). We are currently performing follow-up studies by typing additional markers in these regions. This study illustrates the potential utility of using murine models of anxiety phenotypes to map loci that may influence human anxiety disorders.

Program Nr: 2662 from the 1999 ASHG Annual Meeting

Sib-TDT analysis of human TGF-b1 gene SNPs in female twins: Evidence for linkage and association with blood pressure at the codon 263 polymorphism. *H. Snieder[1], RW. Keen[1], H. Molloy[2], F. Gibson[2], MC. Chiano[2], AJ. MacGregor[1], TD. Spector[1]*. 1) Twin Research Unit, St.Thomas' Hospital, London, UK; 2) Gemini Research Ltd., Cambridge, UK.

Recent evidence suggests that human transfoming growth factor b1 (TGF-b1) is a potential candidate gene for hypertension. This study's aim was to investigate the association between blood pressure and 6 SNPs within the TGF-b1 gene in a large population based sample of female twins. A sib-transmission disequilibrium test (S-TDT) for quantitative traits was used to control for spurious association due to population stratification and/or admixture. Full clinical data was available on 1,598 dizygotic (DZ) female Caucasian twins (799 pairs), mean age 47.3 (SD: 11.4) yrs. Systolic (SBP) and diastolic (DBP) blood pressure was measured twice (OMRON HEM713C). SNPs within the TGF-b1 gene were identified using either ABI TAQMAN or a PCR-based RFLP. Association analysis and the S-TDT were performed using structural equation modelling with the statistical software Mx. In total, 6 polymorphisms were assessed: 2 promoter SNPs (-800, -509), 3 exon SNPs (codon10, codon25, codon263), and 1 intronic SNP (Intron 5). Allele frequencies were: 0.08 (-800), 0.31 (-509), 0.38 (codon10), 0.07 (codon25), 0.03 (codon263) and 0.25 (Intron5). The codon263 showed a significant association with both SBP (p=0.022) and DBP (p=0.013), with higher values in heterozygote carriers (n=54) of the C®T substitution (carriers: 129.4/81.7, non-carriers: 123.4/77.7). Homozygote carriers were absent in our sample. The size of the codon263 polymorphism effect on blood pressure only slightly reduced after adjusting for age and use of antihypertensive medication. Results for the S-TDT confirmed the observed associations (before adjustment: p=.007 and p=.058; after adjustment for antihypertensive medication: p=0.006 and p=0.029 for SBP and DBP respectively), making it unlikely that above results are due to population stratification and/or admixture. This study has shown linkage in the presence of association between the TGF-b 263 polymorphism and blood pressure levels with carriers showing a 6 and 4 mmHG increase in SBP and DBP respectively.

Program Nr: 2663 from the 1999 ASHG Annual Meeting

The role of the 5-HT2A receptor gene in psychosis. *M.S. Sodhi[1], K.J. Aitchison[2], R.W. Kerwin[2], P. Sham[2].* 1) Molecular Neuropathology, Dept Psychiatry, Oxford University, England; 2) Dept Psychological Medicine, Inst of Psychiatry London.

Dysfunction of the 5-HT2A receptor has been implicated in the aetiology and treatment of psychosis by evidence from pharmacological, anatomical and genetic investigations. Many of the genetic association studies published have been conducted on small samples and have produced conflicting data. In this investigation we have combined several independent case control association studies of the 5-HT2A T102C polymorphism by meta-analysis to increase statistical power and to reach more reliable conclusions.

Firstly we failed to replicate the significant association found in a Japanese sample between the 5-HT2A T102C polymorphism and affective disorder[1]in British patients with unipolar disorder and controls(n=118, c^2=0.51, ns). Meta-analysis of the two studies detected increased frequency of the 5-HT2A C102 allele in patients (c^2=4.04, 1df, p=0.04). By contrast, no association was found with bipolar disorder after meta-analysis of 5 published studies (c^2=1.90, ns).

Case control association studies of this marker in schizophrenia have been numerous and have produced some evidence of association[2]. However meta-analysis including all 20 studies performed to date shows no association, although when white Caucasian samples were considered separately (n=12), an excess of the 5-HT2A C102 allele was observed in patients (c^2=7.08, 1df, p=0.007).

In conclusion, allelic variation of the 5-HT2A receptor gene is associated with schizophrenia in white Caucasians and with unipolar disorder, although not with bipolar disorder. The function of this silent genetic change remains to be elucidated and it is possible that another mutation in linkage disequilibrium with it is responsible for the effects observed.

[1]Zhang et al,(1997) Biol Psychiatry, 41(7),768-773

[2]Williams et al., (1997) Lancet,349(9060),1221.

Program Nr: 2664 from the 1999 ASHG Annual Meeting

Variant Alzheimer's Disease with Spastic Paraparesis: Clinical Characterization. *M. Somer[1], A. Verkkoniemi[2], H. Kalimo[3], L. Myllykangas[2,4], R. Crook[4], J. Hardy[4], J.O. Rinne[3], M. Viitanen[5], M. Haltia[2].* 1) Department of Medical Genetics, The Family Federation of Finland, Helsinki, Finland; 2) Departments of Pathology and Clinical Neurosciences, Helsinki University Central Hospital, Helsinki, Finland; 3) Departments of Pathology and Neurology, Turku University Hospital, Turku, Finland; 4) Neurogenetics Laboratory, Mayo Clinic Jacksonville, Jacksonville, FL; 5) Division of Geriatric Medicine, Karolinska Institutet, Huddinge Hospital, Huddinge, Sweden.

We have identified a Finnish family with 23 members in four generations affected with presenile dementia and spastic paraparesis. This variant Alzheimer's disease is due to deletion of exon 9 of the presenilin 1 gene. Neuropathological analysis shows cortical "cotton wool" plaques immunoreactive for the b amyloid peptide but lacking congophilic cores. The mean age at onset (±SD) is 50.9±5.2 years (range 40 to 61 years). All patients have memory impairment with deficits in immediate and delayed recall together with other cognitive deficits. Dementia appears simultaneously with or is preceded by walking difficulty due to spasticity of the lower extremities. However, minority of patients have dementia without spastic paraparesis. Clumsiness of hands and dysarthria suggesting cerebellar involvement is common. MRI of head reveals temporal and hippocampal atrophy, and PET shows bilateral temporo-parietal hypometabolism. Spastic paraparesis and/or brisk tendon reflexes of lower extremities combined with dementia should alert the clinician to consider this variant Alzheimer's disease.

Program Nr: 2665 from the 1999 ASHG Annual Meeting

Imprinting study of Prader-Willi syndrome with methylation-specific PCR (MSPCR). *M. Song, L. Li, J. Fu, X. Li, G. Lu.* Human Reproductive Engineering Laboratory, Hunan Medical University, Hunan, Changsha, P.R.China.

OBJECTIVE: Methylation-specific PCR (MSPCR) assay for genomic imprinting study was established and a case of a 14-year-old boy with typical symptoms associated with the Prader-Willi syndrome (PWS) was detected by this method, and confirmed by Southern blot methylation analysis. METHODS: The 5' CpG island of the SNRPN gene, candidate gene for PWS, is methylated and unmethylated on the maternally and paternally inherited chromosome 15, respectively. MSPCR is based on sodium bisulfite treatment of DNA, which converts unmethylated, but not methylated cytosine residues to uracil, and PCR primers specific for the maternal and the paternal allele. Methylation analysis by Southern blot was used to validate the MSPCR result. High-resolution banding (HRB) chromosome analysis was used to rule out deletion of 15q11-q13. RESULTS: Bisulfite-modified DNA from this patient amplified only with methylated allele-specific primer pair, showing maternal imprinting. This MSPCR result was confirmed by Southern blot methylation analysis. MSPCR and PW71B methylation studies showed an abnormal pattern consistent with the diagnosis of PWS. High-resolution banding analysis failed to reveal any deletion of 15q11-q13. CONCLUSIONS: It is known that methylation-specific PCR can detect all presently testable causes of PWS in an efficient first step for stepwise diagnostic testing. We proved here again that MSPCR is an effective and reliable method for the detection of methylation in genetic disorders of imprinting compared with time-consuming Southern blot methylation analysis.

Program Nr: 2666 from the 1999 ASHG Annual Meeting

Cardiovascular disease genetic risk assessment with a multilocus assay. *C. Stranieri[1], G. Malerba[1], E. Trabetti[1], D. Girelli[2], O. Olivieri[2], R. Corrocher[2], G. Gandini[3], S. Cheng[4], M. Grow[4], H. Erlich[4], P.F. Pignatti[1]*. 1) Section of Biol. and Genet., DMIBG, Univ. Verona, Italy; 2) Dpt. of Exp. and Clin. Med., Univ. Verona, Italy; 3) Blood Center, Hospital of Verona, Italy; 4) Dpt. of Human Genetic, Roche Molecular System, Alameda, CA, USA.

Several genes may be implicated in cardiovascular disease (CVD) risk, together with environmental factors. A multilocus genotyping assay was set up including 15 genes (68 alleles) related to various aspects of cardiovascular physiology: lipid metabolism (ApoE, ApoB, ApoCIII, CETP, LPL, PON), hypertension (ACE, ATIIR, AGT), homocysteine metabolism (CBS, MTHFR), thrombosis (Factor V, Fibrinogen, GPIIIa), and leukocyte adhesion (ELAM), (Cheng et al, Clin. Chem. Lab. Med. 1998; 36:561-66). A series of Italian patients presenting with coronary artery occlusion (CAD+, n=487) or not (CAD- mainly affected by valvular heart disease, n=261) detected by coronarography performed in the same clinic, were selected (Blood 1998; 91:4158-63). Also, blood donors from the same town (Verona, n=108) or a town located 100 Km away (Ferrara, n=138) were selected, persons whose grandparents were all born in the same region. Allele and genotype frequencies were determined in all persons for all polymorphisms in each cohort. No significant difference was obtained between blood donor groups. A significant difference was obtained for ApoCIII T(-455)C genotype distribution between CAD+ and CAD- patients (T/T: 0.37 v. 0.46; T/C: 0.44 v. 0.45; C/C: 0.19 v. 0.09, respectively. P=0.001). A significant difference was obtained in the frequency of the ATIIR A1166C polymorphism genotypes between CAD+ and blood donors in males (A/A: 0.54 v. 0.4; A/C: 0.4 v. 0.5; C/C: 0.06 v. 0.1 respectively. P=0.004) and for the CETP I405V between CAD- and blood donors in females (I/I: 0.39 v. 0.6; I/V: 0.58 v. 0.3; V/V: 0.03 v. 0.1 respectively. P=0.003). No other difference was obtained in any other comparison performed. The data indicate a possible correlation between the ApoCIII gene mutation and coronary atherosclerosis. Further analysis will be performed with the use of other clinical and laboratory data which have been collected.

Program Nr: 2667 from the 1999 ASHG Annual Meeting

Polymorphism of NACP-Rep 1 in patients with Parkinson's disease. *E.K. Tan[1], T. Matsuura[1, 2], S. Nagamitsu[1, 2], M. Khajavi[1, 2], J. Jankovic[1], T. Ashizawa[1, 2].* 1) Neurology, Baylor College of Medicine, Houston, TX; 2) Neurology Service, VA Medical Center, Houston, TX.

Background: Missense mutations in the alpha-synuclein gene has been identified in certain autosomal dominant Parkinson's disease (PD) families. Recently, Kruger et al have demonstrated that a polymorphism in the promoter region (NACP-REP 1) of this gene is associated with susceptibility to sporadic PD in German population (Ann Neurol 1999;45:611-7). Such an association has not been studied in American families.

Objective: To determine if there is an association between polymorphism of NACP-Rep 1 and susceptibility to PD in American patients.

Methods: This study includes 104 patients diagnosed to have idiopathic PD (58 gave positive family history of the disease) using standardized clinical criteria and 125 controls without the disease. The allele status in the promoter region of the gene was determined by a PCR-based method. Four different alleles were identified; 259bp, 261bp, 263bp and 265bp (novel allele).

Results: The allele frequencies of PD patients and controls were; 259bp (0.22 vs 0.33, $p<0.007$), 261bp (0.70 vs 0.56, $p<0.005$), 263bp (0.08 vs 0.10) and 265 (0.005 vs 0.004) respectively. Genotype frequencies of PD and controls were; 259/259bp (0.01 vs 0.10, $p<0.002$), 259/261bp (0.37 vs 0.35), 261/261bp (0.44 vs 0.33), 259/263bp (0.06 vs 0.09), 261/263bp (0.12 vs 0.11), 261/265bp (0.01 vs 0), 259/265 (0 vs 0.01) respectively. There were no differences in the allele distribution in PD patients with and without a family history of the disease.

Conclusions: There are significant differences in the allele distribution between PD patients and controls. Allele of 261bp is associated with increased PD susceptibility, whereas the 259bp is associated with decreased PD susceptibility. This contrasts with the findings in the German sporadic PD population where 263bp was associated with increased susceptibility. Thus these results questions the significance of the contribution of this polymorphism to NACP-Rep 1 gene function in susceptibility of PD.

Program Nr: 2668 from the 1999 ASHG Annual Meeting

The role of RNA CUG triplet repeats in Myotonic Dystrophy. *L.T. Timchenko[1], X. Lu[1], A-S. Lia-Baldini[1], Z-J. Cai[1], J. Chiu[1], N.A. Timchenko[2].* 1) Dept Cardiology/Medicine, Baylor Col Medicine, Houston, TX 77030; 2) Dept of Pathology and Huffington Center, Baylor Col Medicine, Houston, TX 77030.

Myotonic dystrophy (DM) is a neuromuscular disease characterized by multiple defects including myotonia, skeletal muscle weakness, brain dysfunction, and cardiac and endocrine abnormalities. Mutation responsible for DM is an unstable CTG trinucleotide repeat located in the 3' untranslated region of the myotonin protein kinase (DMPK) gene. We proposed that trinucleotide repeats in DMPK gene act at the RNA level sequestering specific RNA-binding proteins. In agreement with this hypothesis, a CUG-binding protein, CUGBP1, has been isolated. Immunoanalysis and library screening identified additional RNA binding proteins that can bind to CUG repeats: ETR-3 and apoptosis-related protein. Cardiac specific protein, ETR-3, binds to CUG repeats similar to CUGBP1. Binding affinity of the apoptosis-related protein is under investigations. Analysis of CUGBP1 expression showed that this protein is affected in DM disease and in cultured cells by expression of RNA CUG repeats. Northern analyses suggest that different members of CUG-binding proteins might function in a tissue specific manner. While CUGBP1 is expressed in all tissues with the highest levels in skeletal muscle and heart, ETR-3 mRNA is abundant in heart tissue. The apoptosis-related protein has been detected in the brain and heart. Tissue-specific expression of CUG-binding proteins suggests that they might have different functions in RNA processing regulating different RNA targets. Our data show that CUGBP1 is involved in the regulation of both translation and splicing of cardiac and liver-specific RNAs. Thus, these results demonstrate the existence of a family of RNA binding proteins that might be affected by expansion of CUG repeats in DM. Functional analysis of members of the CUG-binding proteins family and identification of their native RNA targets would help to elucidate molecular basis for DM pathogenesis.

Program Nr: 2669 from the 1999 ASHG Annual Meeting

RNA binding proteins involved in Myotonic Dystrophy. *G. Tiscornia, M. Mahadevan.* Dept Medical Genetics, Univ Wisconsin Madison, Madison, WI.

Myotonic Dystrophy is a complex, multisystemic inherited neuromuscular disorder. The DM mutation is a CTG expansion in the 3' untranslated region of the DMPK gene. The mechanism by which this mutation causes disease is unclear. It has been suggested that altered cellular RNA metabolism caused by the mutant DMPK mRNA contributes to DM pathophysiology. The mutant DMPK transcript forms nuclear aggregates of RNA in patient cells. We have undertaken a search for RNA-binding proteins interacting with the DMPK 3'UTR RNA. Radiolabeled RNA probes consisting of the normal or mutant DMPK 3'UTR were used to perform UV crosslinking assays in HeLa, C2C12 and fibroblast protein extracts. We have identified several RNA protein interactions which are specific for the DMPK 3'UTR RNA, as indicated by competition assays using excess amounts of unlabeled homologous or heterologous RNAs. By using radiolabeled RNAs composed of various segments of the 3'UTR, we initially mapped the binding sites of the interacting proteins to a 500 nucleotide region spanning from (but not including) the CUG repeats to the DMPK polyadenilation site. Use of smaller riboprobes within this sequence has led to the identification of two regions of 70 and 100 nucleotides respectively that account for the binding activity of the interacting proteins. We are currently working on identifying the proteins involved in the interaction and investigating what role they play in transcript metabolism and DM pathophysiology. We have determined that the interactions are unaffected by expansions of 130 CUG repeats, that there are no differences in binding activity of protein extracts from normal vs. DM cell lines and that a reporter construct spliced to a DMPK 3'UTR lacking the binding sites does not result in significant changes in reporter activity. Nontheless, a cell culture model developed in our lab has established that sequences downtsream of the repeats play a role in cell fusion of mouse myoblasts, suggesting a possible role for the RNA-binding proteins we have detected in that phenotype.

Program Nr: 2670 from the 1999 ASHG Annual Meeting

Dendritic spine maturation is delayed in cultured hippocampal neurons of the Fmr1 knockout mouse. *E.R Torre, S.T. Warren.* Howard Hughes Medical Institute and Departments of Biochemistry, Genetics and Pediatrics. Emory University School of Medicine. Atlanta, Georgia 30322.

Fragile X syndrome is a frequent cause of inherited mental retardation and is due to the absence of the Fmr1 protein, FMRP. The cognitive deficit in patients has been associated with an apparent compromise of synaptic structure found in postmortem cortices and characterized by long, thin, and tortuous spines. However, other forms of mental retardation have also been shown to exhibit these features. Therefore it remains unclear if the observation of abnormal spine morphology in fragile X syndrome is a direct consequence of FMRP loss or a general result of cognitive deficits which limit adaptation and interaction. To directly answer this question, we analyzed the development of dendritic spines of cultured primary neurons derived from the Fmr1 knockout mouse. Hippocampal brain cells from E16 wild type (WT) or Fmr1 knockout (KO) mice were plated on glass coverslips and cultured for 14, 21, and 35 days. Neuronal morphology was assessed by staining with the lipophilic dye, DiI, and dendritic spines were identified by colocalization with the presynaptic marker synaptophysin. By day 14, neurons were profusely innervated and dendritic spines were easily visualized in both WT and KO neurons. The mean length of dendritic spines for KO neurons was 2.41 ± 0.3mm (1462 spines from 16 cells) as compared to 1.6 ± 0.1mm (2417 spines from 23 cells) for the WT neurons. Further analysis reveals that the increase in length of KO spines reflects a decrease in the number of spines within the normal range size for that age (1 to 2mm) and a two-fold increase in the frequency of spines ranging between 2 and 4mm. Differences between the KO and WT spines are most manifest at 20 days in culture and almost disappear by day 35. Synaptic contacts transform long, thin filopodias into short, mature spines with stubby and mushroom shapes. Hence, these data suggest: a) the loss of FMRP influences synaptic plasticity and, in particular, delays synapse maturation; b) since cultured neurons presumably grow under identical environmental conditions, the spine differences are directly due to the loss of FMRP.

Program Nr: 2671 from the 1999 ASHG Annual Meeting

The use of cDNA-representational difference analysis (cDNA-RDA) to characterize expression differences between Proteus Syndrome paired tissue samples. *S.J. Vacha[1], S.H. Shin[1], P.S. Meltzer[3], M.M. Cohen, Jr.[2], L.G. Biesecker[1]*. 1) Genetic Disease Res Branch, NHGRI, NIH, Bethesda, MD; 2) Dalhousie University, Halifax, Nova Scotia, Canada; 3) Cancer Genetics Branch, NHGRI, NIH,Bethesda, MD.

Proteus syndrome (PS) is a rare, sporadic disorder characterized by the progressive overgrowth of various regions of the body. The range and severity of features, including hyperostoses, connective tissue nevi, macrodactyly, hemangiomas, and tumor predisposition, is highly variable between patients. It is hypothesized that this disorder is due to a postzygotic somatic mutation that is lethal in a non-mosaic state. To date, there are no molecular data for PS regarding the gene, type of mutation, or chromosomal location. Preliminary genomic experiments (comparative genomic hybridization, microsatellite analysis, representational difference analysis) comparing normal vs. abnormal tissues from within affected individuals did not detect aberrations. For this reason, cDNA-RDA was performed to characterize gene expression differences between paired fibroblasts of two affected patients. A positive control was successful in enriching for 3' Gli3 cDNA in a comparison between full-length Gli3-transfected 293 cells and truncated Gli3-transfected 293 cells. From each RDA difference product, 500 enriched clones were sequenced and further confirmed by colony blot array as well as Southern and Northern blot analysis. To characterize these clones we have employed an arraying approach that validates or rejects clones that are generated by this procedure. First, we array a large sample of clones from one RDA and then probe that array with a clone pool from the complementary experiment (where tester and driver were reversed) or with the same experiment performed on tissues from other Proteus patients. The second array approach is to use RNA from affected and unaffected cell lines to screen a 10K expression array and use these clones to probe the above noted arrays. These procedures should greatly facilitate the analysis of large numbers of cDNA clones in this and other disorders.

Program Nr: 2672 from the 1999 ASHG Annual Meeting

Role of Polymorphisms in Parathyroid Hormone Receptor 1, Transforming Growth Factor Beta 1 and Estrogen Receptor in the Genetics of Osteoporosis. *A.M. Valdes[1], M. Bouzyk[1], E. O'Brien[1], D. Gale[1], S. Panchal[1], M. Barnes[1], S. Ralston[2], D. Reid[2], A. Rut[1], N. Spurr[1].* 1) Smithkline Beecham Pharmaceuticals Harlow, UK; 2) Dept Medicine and Therapeutics, University of Aberdeen, UK.

Osteoporosis is characterized by a reduction in bone mass affecting one in three postmenopausal women. We describe the effects of three genes along the bone remodeling pathway in bone mineral density (BMD) and rate of bone loss. We tested two microsatellite markers near estrogen receptor (ESR1; 6q25) D6S440 and D6S441, a non-coding SNP located in exon 7 of the PTH receptor 1 (PTHR1; 3p22-p21.1), and a SNP located in intron 5 of the transforming growth beta factor 1 gene (TGFB1; 19q13). Estrogen influences bone growth and metabolism; TGFB1 regulates osteoblast and osteoclast activity and modulates bone matrix synthesis; PTH is a major regulator of extracellular calcium homeostasis. The cohort studied consisted of 554 healthy Scottish women (age range 51-57, mean =54.2) Genotypes were tested for association with baseline spine and hip BMD and rate of change measured as percent of BMD loss (or gain) per year. The latter was stratified by use of hormone replacement therapy (HRT). The average time span between BMD measures was 6.7 years. The genotype at the two markers flanking ESR1 accounted for 3.8% of variance in hip BMD ($p< 0.03$) and 6.7% of spine BMD ($p< 0.002$). Significant effects of genotypes on spine ($p<0.002$) and hip ($p<0.01$) rate of change were also found. TGFB1 affected BMD at both hip ($p<0.02$) and spine ($p<0.05$) and accounted for less than 1% of the variance. Individuals carrying genotype TT at PTHR1 not treated with HRT had a greater loss of spine BMD than CT or CC individuals ($p< 0.03$), whereas individuals on HRT had the same rate of change. The interaction effect with HRT response was statistically significant ($p<0.03$). PTHR1 genotype explained only 2% of the variance of spine rate of change. ESR1 and TGFB1 together account for up to 10.2% of the variance in spine BMD and 8.5% of the hip BMD. Some of this appears to come from the gene-gene interaction between ESR1 and TGFB1 ($p<0.05$). We discuss the role of these genes in susceptibility to osteoporosis in the normal population.

Program Nr: 2673 from the 1999 ASHG Annual Meeting

Using Late-Onset Alzheimer's Disease (LOAD) as a Model to Study the Application of Single Nucleotide Polymorphisms (SNPs) for Complex Disease Association Studies: Genotyping and Analysis of 65 SNPs in a 4-Megabase Region Surrounding Apolipoprotein E (APOE). *J.M. Vance[1], E.R. Martin[1], J.R. Gilbert[1], A.R. Rogala[1], A. Afshari[2], B.D. Slotterbeck[1], J.M. Grubber[1], A.M. Saunders[1], J. Riley[3], I. Purvis[3], D.E. Schmechel[1], P.M. Conneally[4], E.H. Lai[2], M.A. Pericak-Vance[1], A.D. Roses[2]*. 1) Center for Human Genetics, Duke University Medical Center, Durham, NC, USA; 2) Glaxo Wellcome, Inc., Research Triangle Park, NC, USA; 3) Glaxo Wellcome, Inc., London, UK; 4) Dept Medical Genetics, Indiana University Medical Center, Indianapolis, IN, USA.

Considerable discussion has occurred in recent years concerning the use of SNPs and association to find loci contributing to common, complex genetic disorders. However, little actual data is available to investigate this approach. APOE is a proven susceptibility gene for LOAD. Thus, it presents an excellent model to actually test and develop methodology for genotyping and data analysis, as well as many of the proposed assumptions affecting this model. In addition, it can be used to help predict some of the parameters likely to be needed in this gene mapping approach. We have previously studied 10 SNPs surrounding the APOE locus and found that the region of disequilbrium for LOAD and APOE was relatively small, but detectable. Thus, to further map this region and develop data for investigation of methodology for SNP data analysis and SNP genotyping, we have genotyped 65 additional SNPs surrounding APOE. 877 individuals consisting of LOAD patients and age-matched controls were genotyped using Taqman® methodology. Samples include both case controls as well as LOAD families, allowing sibling-based allelic association techniques to be applied as well. Genotyping was performed using quality control measures (samples duplicated in a blinded fashion) to provide estimates of the accuracy of the handling and genotyping methods. Association studies, maps and genotyping results will be presented.

Program Nr: 2674 from the 1999 ASHG Annual Meeting

Transmission on a fragile X full mutation through a normal transmitting male. *K.A. Ventura[1], L.M. Fraer[1], E.E. Smith[1], A.E. Merrill[2], K.J. Treat[2], Z. Wang[2], B.A. Allitto[2], J.M. DeMarchi[2], W.A. Hogge[1]*. 1) Dept Genetics, Magee Womens Hosp, Pittsburgh, PA; 2) Genzyme Genetics, Framingham, MA.

The Fragile X premutation has been thought to be unstable during female, but not male, transmission. We report a family in which a Fragile X full mutation was inherited through an unaffected male with a premutation. The female proband presented at 12 weeks gestation because of mental retardation in a sister and three maternal cousins (two boys and one girl). The cousins had been evaluated previously at another institution, and chromosome analyses were normal. Chromosome studies and Fragile X DNA analysis were performed on the proband and her sister. The proband was found to be 46,XX and negative for Fragile X with 20 and 38 CGG repeats. Her sister was found to be 46,XX with a full mutation (38 and 600 repeats) for Fragile X syndrome. Testing was performed on other maternal family members: the proband's mother and maternal aunt were found to be full mutation (abnormal methylation) carriers, with 20 and 300, and 30 and 267 repeat alleles, respectively; an uncle had 20 repeats; the grandmother had 20 and 30 repeats. Therefore, the premutation did not originate from the grandmother as expected. Fragile X DNA analysis of the grandfather, however, showed normal methylation, and a repeat size of 59, with a faint but reproducible smear from 59 to greater than 200 repeats detected by PCR. The PCR result and the inheritance pattern suggest instability of the 59-repeat allele and/or possible germline mosaicism for a full mutation. Since this family study indicates a transmitting male passed on a full mutation to at least two daughters, counseling of families with suspected Fragile X syndrome on the paternal side should be undertaken with caution.

Program Nr: 2675 from the 1999 ASHG Annual Meeting

Identification of genes at translocation breakpoints on chromosome 7q31 in autistic individuals. *J.B. Vincent[1], J.A. Herbrick[1], H.M.D. Gurling[2], S.W. Scherer[1],* .. 1) Department of Genetics, The Hospital for Sick Children, Torornto, ON, Canada; 2) Molecular Psychiatry Laboratory, Windeyer Institute of Medical Sciences, University College London Medical School, London, UK.

Autism is a severe and debilitating childhood-onset developmental disorder, characterized by impaired communication and social interaction coupled with preoccupations, repetitive behaviours, circumscribed interests and activities. The prevalence of typical autism has been estimated to be between 0.77 and 5.6 per 10,000, while a broader phenotype of autism spectrum disorders have a population prevalence of 5 in 1000. A strong genetic component is believed to be involved. The mode of genetic transmission for autism is unclear, however a complex, non-Mendelian inheritance pattern is suggested. Results from the first genome-wide scan for linkage to autism have recently been published. Regions on six chromosomes (4, 7, 10, 16, 19 and 22) were identified with multipoint maximum LOD scores (MLS) >1, with a most significant MLS of 3.55 on 7q. Initial results from autism genome scans by several other groups also implicate the 7q region. Linkage results for a severe speech and language disorder have also implicated a locus (SPCH1) on the same region of 7q. We have identified a number of autism patients and individuals with developmental or language delay with translocations or deletions involving 7q31. We have localised the breakpoint for one autism patient with a translocation t(7;13)(q31.2;q21) to a BAC clone within the critical region for linkage for autism, and a transcript which spans the breakpoint has been cloned. The function of this novel gene is unknown but characterisation is currently underway. A mutation search in this gene is underway in autistic individuals from multiplex families which share haplotypes around this gene.

Program Nr: 2676 from the 1999 ASHG Annual Meeting

Abnormalities in fibrillin-1-containing microfibrils in fibroblast cultures from patients with scleroderma. *D.D. WALLIS[1], F.K. TAN[1], C. KIELTY[2], F.C. ARNETT[1], D.M. MILEWICZ[1]*. 1) INT MED UNIV OF TX MS HOUSTON, HOUSTON, TX; 2) UNIV OF MANCHESTER UK.

Fibrosis of skin and viscera that is the hallmark of scleroderma (SSc) is produced by activated fibroblasts. The *Tsk* mouse model of SSc results from a fbn1 mutation which encodes fibrillin-1, a major component of microfibrils (MF) in the extracellular matrix (ECM). Evidence that *FBN1* alterations contributes to human SSc comes from studies of Choctaws, where a *FBN1* haplotype is associated with SSc. Mutations in *FBN1* cause Marfan syndrome (MFS) in a dominant negative pathogenesis where the mutant fibrillin-1 (fib-1) disrupts MF formation. Therefore, studies were undertaken using established methods to determine if there was abnormal fib-1 function or MF structure in skin fibroblasts explanted from 12 SSc patients (including 2 Choctaws and 2 siblings). Pulse-chase studies indicated diminished amounts of fib-1 in the ECM in 6 of 12 SSc strains compared to controls (n=14). Five strains had no recognizable MF by rotary shadow electron microscopy (R-EM) and 7 had diminished MF. Immunoblotting of high molecular weight ECM fractions from 2 Choctaw SSc fibroblast strains with antibodies for fib-1 and type VI collagen confirmed the lack of intact fib-1-containing MF; the amount of type VI collagen MF was similar to control cells. Immunofluorescence of cultures (12 SSc, 3MFS, 5 controls) demonstrated that the control and SSc fibroblasts displayed a prominent meshwork of fib-1 MF. MFS fibroblasts showed diminished MF, consistent with a dominant negative effect of the mutant fib-1 on MF formation. Four unaffected relatives of a SSc patient were biopsied and 2 of these cultures showed diminished fib-1 in the ECM with pulse-chase analysis. These results indicate that SSc fibroblasts can assemble MF in contrast to MFS fibroblasts. The diminished amounts of fib-1-MF found in the ECM of SSc cultures by metabolic labeling or R-EM implies that the assembled MF are abnormal and degrade with biochemical manipulation. Models are proposed to link the observed MF instability to the activated fibroblasts. Furthermore, the instability of the MF may be genetically determined based on the family studies.

Program Nr: 2677 from the 1999 ASHG Annual Meeting

Identification of a recombination hotspot on chromosome 15q11-13 in children with autism. *C.H. Wang[1,3,4], E. Villaca-Norat[1], M.B. Miller[2], B.D. Papendick[1], D.K. Gavrilov[3], R.E. Hillman[4], J.H. Miles[4].* 1) Neurology; 2) Psychology; 3) Biochemistry; 4) Child Health, Univ Missouri, Columbia, MO.

Autism is a complex genetic disorder characterized by repetitive stereotypic behaviors and impaired communication and social interaction. Recent cytogenetic and molecular studies indicate that DNA rearrangements within the chromosome 15q11-13 region may contribute to the pathogenesis of autism. We characterized the genomic arrangement of this region in 126 autism families using eleven microsatellite markers spanning about 3.0 Mb interval. Samples from four autism probands were trisomic for several 15q alleles. Karyotype analysis revealed supernumerary isodicentric 15q13-pter in 4 probands. One included a supernumerary derivative 15 chromosome from a balanced maternal 4/15 translocation. Of the 122 remaining families 31 were informative for haplotype analysis in this region yielding 60 informative meioses in transmissions to affected offspring. In six (10%) of these meioses, crossovers were observed between markers D15S986 and D15S1234 (an interval of less than 200 Kb). Four of these crossovers occurred on maternal chromosomes and two on the paternal chromosome. Of 179 meioses in control families, 1 (0.6%) demonstrated meiotic recombination within this interval. This region of frequent autism- associated recombination is distinctly different from the meiotic breakpoints observed in the control groups which were located more telomerically. We propose that meiotic recombination in chromosome 15q11-13 may result in genomic rearrangement and contribute to the pathogenetic etiology in children with autism.

Program Nr: 2678 from the 1999 ASHG Annual Meeting

Sulfonylurea Receptor Gene Polymorphism is Associated with Non-insulin Dependent Diabetes Mellitus(NIDDM) in Chinese Population. *H. Wang, L. Ji, X. Han.* Department of Endocrinology and Metablism, People's Hospital, Beijing Medical University, Beijing, P.R.China.

Non-insulin dependent diabetes mellitus(NIDDM) is a common polygenic disease, and deficiency in insulin secretion as well as in insulin utilization are involved in the pathogenesis of NIDDM. As a hypoglycemic drug, sulfonylurea has been widely used for the treatment of NIDDM. Sulfonylurea receptor gene was cloned in 1995 and its mutations were shown to be associated with familial persistent hyperinsulinemic hypoglycemia of infancy. In addition, polymorphisms in intron 24(-3t-3c) and exon 22 (T761T) of SUR gene were found strongly associated with NIDDM in Caucasians population , suggesting the SUR gene may contribute to genetic susceptibility of NIDDM. To study the role of SUR gene in the pathogenesis of NIDDM in Chinese population, we test the polymorphisms of the SUR gene in intron 24 and exon 22 by PCR-RFLP method in 86 NIDDM patients with at least two first degree diabetic relatives and 148 normal control subjects. The results show that the frequency of " -3 c "allele of intron 24 in NIDDM patients is significantly increased comparing with that in the control subjects (68.02% vs. 55.41% , P=0.007), and the frequency of the "-3c/-3c" genotype of intron 24 in the NIDDM group is also significantly higher than that in control group.(41.86% vs. 27.7%, P=0.013 ,OR=4. 39, CI: 1.52-12.66). The polymorphism of exon 22 described in the Caucasian population was not detected in this study. In conclusion, the association of the polymorphism of SUR gene with NIDDM in different races suggests that the SUR gene or nearby gene may play an important role in the genetic susceptibility of NIDDM.

Program Nr: 2679 from the 1999 ASHG Annual Meeting

Variable effects of the ApoCIII C-482T variant on insulin and triglyceride levels in different ethnic groups. *D.M. Waterworth[1], F.P. Cappuccio[2], D.G. Cook[2], P.D. Wicks[2], S.E. Humphries[1], P.J. Talmud[1]*. 1) Cardiovascular Genetics, RFUCLMS, London, UK; 2) St George's Hospital Medical School, London UK.

We have previously reported that the ApoCIII C-482T variant modulates insulin and glucose levels after an OGTT in young healthy white men (Circulation. 1999;99:1872-1877). In order to evaluate its effect in different ethnic backgrounds, this variant was investigated in a population-based cross-sectional study in South London which consisted of Whites (n=462), South Asians (n=442) and individuals of African origin (n=462). There was a considerable difference in T-482 allele frequency between the three groups: 0.25(CI 0.22-0.28) in Whites, 0.44(CI 0.41-0.47) in South Asians and 0.71(CI 0.68-0.74) in individuals of African origin. Significant associations with fasting insulin levels were observed in White males (p=0.03) and Black males (p=0.005), but not in South Asian males or any of the female groups. In whites, the group with the genotype CC-482 had the lowest mean insulin levels, while in blacks (the opposite association was observed) the TT-482 group had the lowest levels. No associations were observed with 2-hour post-load insulin in any of the groups. Relationships between C-482T and triglyceride were also investigated and a significant association was found only for white males (p=0.03). In order to clarify these relationships with insulin and triglyceride, linear regression models were constructed separately for each ethnic group and confirmed a relationship between insulin levels and C-482T in Whites (p=0.01) and Blacks (p=0.04) but gave no evidence in Asians (p=0.20). The significant relationship between C-482T genotype and triglyceride in Whites was confirmed (p=0.02) and there was also borderline significance observed for Asians (p=0.05), but there was no evidence in Blacks (p=0.88). This study illustrates the important influence of genetic background when investigating the effects of genetic variation on insulin sensitivity and lipids.

Program Nr: 2680 from the 1999 ASHG Annual Meeting

UBE3A Mutations in Angelman Syndrome: Types, Hotspots, and Origins. *H. Webber, J. Wagstaff.* Genetics Division, Children's Hospital, Boston, MA.

We have found mutations in the UBE3A ubiquitin-protein ligase gene in 28 sporadic or familial Angelman syndrome (AS) cases lacking 15q11-q13 deletions, chromosome 15 UPD, or imprinting defects. We and others (Fang et al., Hum. Mol.Genet. 1999, 8:129-135) have found a much higher frequency of mutations in familial cases (75-80%) than in sporadic cases (14-23%). Among 28 mutatations detected by SSCP, we have found 17 small insertions or deletions causing frameshifts, 3 mutations to stop codons, 3 single-amino-acid deletions or duplications, 2 splice site mutations, 2 missense mutations, and 1 mutation predicted to cause a 7-amino-acid deletion. We found multiple mutations at 2 sites: the same de novo 5-bp duplication in exon 16 was detected in 3 individuals, and 3 different single-amino-acid deletion or duplication mutations were found within an 8-bp region of exon 15. Among 14 mutations that could be traced to their origins, 9 apparently arose in the maternal germline; 2 arose in the paternal germline; 2 arose in females with somatic and germline mosaicism; and 1 arose in a mosaic male. The 2 missense mutations lead to nonconservative amino acid changes in the region of UBE3A required for E6-dependent association with p53. These missense mutations should prove useful in determining which UBE3A substrates are relevant to the pathogenesis of AS.

Program Nr: 2681 from the 1999 ASHG Annual Meeting

A comprehensive screen for genetic variation in the transforming growth factor b1 gene in multiple sclerosis.
B.G. Weinshenker, D. Hebrink, O. Kantarci, E. Atkinson, C.T. McMurray. Mayo Clinic/Mayo Foundation, Rochester, MN.

Background: Multiple sclerosis (MS) is a complex genetic disease. The course of MS is highly variable; the biological determinants of the variability are unknown. We evaluated transforming growth factor beta 1 (TGFb1) as a candidate gene for MS susceptibility and course. TGFb1 is a TH2 immunoregulatory cytokine that opposes the effects of pro-inflammatory TH1 cytokines. TGFb1 administration ameliorates and neutralizing antibodies to TGFb1 exacerbate experimental allergic encephalomyelitis, a model for MS. TGFb1 is encoded on chromosome 19q13, a region implicated in MS susceptibility by three independent whole genome scans. Methods: We scanned the promoter region and exons 1-7 and their splice junctions by dideoxyfingerprinting (ddF) or restriction endonuclease fingerprinting (REF). These mutation detection methods are hybrids of either sequencing (ddF) or multiple independent restriction digests (REF), combined with non-denaturing electrophoresis to detect single strand conformational polymorphism. We tested variants of sufficient frequency for association with disease susceptibility and severity. Patients were categorized for MS-related disability from 1 (most severe) to 10 (least severe) as previously described (Weinshenker et al. Neurology 1997; 49: 378-385). Controls were matched for age, gender and ethnicity in a ratio of 2:1. Results: We identified six sequence variants, including four in exons and one in the promoter region. Three missense variants were detected: exon 1(Gb_pr X05839) position 2005T®C (L10P) and 2050G®C (R25P); exon 5(Gb_prX05844) position 342 C®T (T263I). We evaluated variants at positions 2005 and 2050 of exon 1, and position -510 in the promoter region. There was no association of the three variants with disease susceptibility, but a trend was found for the -510 variant in the promoter region with disease severity (homozygotes 6.4 ± 1.3; heterozygotes 5.9 ± 1.9; wild type 5.5 ± 1.9; P=0.166). Conclusions: Variants in TGFb1 were not associated with MS susceptibility. A trend to association of a promoter region variant with disease severity was observed.

Program Nr: 2682 from the 1999 ASHG Annual Meeting

Obstetric cholestasis in a woman with no family history of progressive familial intrahepatic cholestasis caused by a novel mutation in the MDR3 gene. *C. Williamson[1,2], N. Weerasekera[1], P.H. Dixon[1], O. Donaldson[1], J. Chambers[1,3], E. Egginton[3], J. Weaver[3], C. Nelson-Piercy[4], M. de Swiet[2], E. Elias[3], D.G. Johnson[1], M.I. McCarthy[1].* 1) Dept. of Medicine, Imperial College, Hammersmith Hospital, London, UK; 2) Institute of Obstetrics and Gynaecology, Queen Charlotte's Hospital, London, UK; 3) Department of Gastroenterology, Birmingham University, Birmingham, UK; 4) Dept. of Obstetrics and Gynaecology, St Thomas' Hospital, London, UK.

Obstetric cholestasis (OC) is a disorder of pregnancy with serious consequences for the mother and fetus, and can cause unexplained third trimester stillbirth. Two pedigrees have been reported with OC in the mothers of children with a subtype of autosomal recessive progressive familial intrahepatic cholestasis (PFIC) with raised gamma glutamyl transpeptidase (GGT). In these pedigrees affected children have homozygous mutations in the multidrug resistance 3 (MDR3) gene, and heterozygote mothers have OC. We therefore investigated a woman with OC and raised GGT, and with no family history of PFIC, for MDR3 mutations. Using previously characterised intronic sequence, together with novel sequence generated from long-range PCR experiments, we designed 27 pairs of primers to amplify the coding exons of the MDR3 gene, together with the intron exon boundaries. DNA sequence analysis of the products revealed a C®A transversion in codon 546 in exon 14. This results in the substitution of the wild type alanine (GCC) with an asparagine (GAC). This DNA base change was confirmed and demonstrated to not be present in 100 normal chromosomes from a panel of parous controls using the restriction enzyme SexAI. Our analysis of the genomic sequence of MDR3 has also revealed a dinucleotide microsatellite polymorphism in intron 15 and a single nucleotide polymorphism in exon 16. These variants will be useful for further linkage and association studies of this gene. This is the first demonstration of a heterozygous missense mutation in the MDR3 gene in a patient with OC with no family history of PFIC. Our analysis indicates that MDR3 mutations may be responsible for OC in a subgroup of women with raised GGT.

Program Nr: 2683 from the 1999 ASHG Annual Meeting

Search for sequence variations in the NDUFA1 Gene of complex I deficient patients. *I. Wittig[1], P. Augstein[2], G.K. Brown[3], T. Fujii[4], A. Roetig[5], P. Rustin[5], P. Seibel[5], D. Thorburn[5], B. Wissinger[5], K. Tensing[5], A. Metspalu[5], E. Lamantea[5], M. Zeviani[5], Y. Goto[5], M. Wehnert[1]*. 1) Institute of Human Genetics, University of Greifswald, Greifswald, Germany; 2) Institute of Diabetes, Karlsburg, Germany; 3) Genetics Laboratory, Oxford, UK; 4) Department of Pediatrics, Shiga Medical Center for Children, Shiga, Japan; 5) Hospital des Enfants-Malades, Paris, France; 6)Research Group Neurobiology and Cell Biology, Dresden, Germany; 7)The Murdoch Institute, Melbourne, Australia; 8)Eye Clinic, Tuebingen, Germany; 9)Institute of Molecular and Cell Biology, Tartu, Estonia; 10)Institute of Neurology, Milano, Italy; 11)Nati.

The NDUFA1 gene is one of the human nuclear genes encoding a subunit of complex I of the respiratory chain that has been cloned and mapped to Xq24. The encoded 70 amino acids (MWFE peptide) predict a hydrophobic and a hydrophilic domain, which are putatively involved in anchoring and orientation of complex I at the inner mitochondrial membrane. MWFE is essential for an active complex I in mammals. The available sequence and mapping information was used to search for a human condition related to sequence variations in the NDUFA1 gene. We scanned for sequence variations in predominantly male patients with LHON (54) and other conditions of complex I deficiencies accompanied with neurodegenerative and / or neuromuscular symptoms including Leighs syndrome (137) from different countries. Under the 191 patients that have been studied by PCR, heteroduplex analysis and direct sequencing, no mutations in the exons (including exon/intron boundaries) and 367 bp of the promoter region have been found so far. Three single nucleotide polymorphisms (SNP), T/C nt at -189, C/G at nt -71 and T/G at nt 1454 were discovered and evaluated by restriction digestion of the PCR products in a German population (frequencies for the frequent alleles are 0.938, 0.512 and 0.963, respectively). As compared to the patient group no significant difference was observed. The polymorphisms seem to be neutral and not related to a disease phenotype caused by complex I deficiency, although population differences in allele frequencies have been found for all SNPs.

Program Nr: 2684 from the 1999 ASHG Annual Meeting

What else NPL linkage scores can tell us about complex diseases. *M. Wjst, T. Immervoll, S. Loesgen.* Institut fuer Epidemiologie, GSF Forsch Umwelt & Gesundh, Neuherberg Munich, Germany .

We report a reanalysis from a genome-wide scan for asthma and related traits in 97 families with at least 2 asthmatic children between age 6 and 18 recruited from several university hospitals in Germany and Sweden. All participants completed a detailed interview (Clin Exp All 1995; 18 Suppl: 482). Microsatellite typing was done in a high throughput laboratory with a panel of 351 markers evenly spaced over the genome with about 10 cM distance. For a multipoint linkage analysis a nonparametric IBD-based statistic was used as implemented in the software package Genehunter. Four identified regions suggestive for linkage to asthma were identified: chr. 2, 6, 9 and 12 (Genomics 1999; 58: 1-8).

For the current analysis NPL score at each region were set to missing if the PIC was less than 0.90 and coded as 1 or 0 for each subject if greater than 80% of the distribution. A logistic regression analysis then included clinical details as age of onset, attack rate, duration, severity and asthma trigger type. The locus on chr.2 seems to promote more an infection type asthma (odds ratio OR 1.4, NS) with longer attack durations (OR 2.0, NS) and less pollen induced asthma (OR 0.7, NS). The loci on chr. 6, 9, 12 are all in favour of pollen induced asthma with OR 4.6 (NS), OR 1.6 (NS) and OR 6.2 (NS) respectively. The patients with linkage at the chr.6 locus patients are less females (OR 0.3, P=0.08), have later disease onsets (OR 1.3, P=0.02) and longer attack duration (OR 2.5, P=0.02). The patients with the chr.12 locus have higher total IgE levels (OR 2.0, P=0.08 for log(IgE) increase). Despite a lack of power in the present study this approach seems to be useful to discriminate clinical subtypes of asthma.

Program Nr: 2685 from the 1999 ASHG Annual Meeting

Atrophin-1, the DRPLA gene product, interacts with a component of nuclear corepressor complexes, and associates with the nuclear matrix. *J.D. Wood[1], K. Duan[1], F.C. Nucifora, Jr.[1], J. Wang[2], Y. Kim[1], G. Schilling[1], J. Lui[2], C.A. Ross[1]*. 1) Division of Neurobiology, Dept. of Psychiatry, Johns Hopkins University, Baltimore, MD; 2) Hematology Branch, NHLBI, NIH, Bethesda, MD.

Dentato-rubral and pallido-luysian atrophy (DRPLA) is one of the family of autosomal dominant neurodegenerative disorders caused by the expansion of a polyglutamine tract. It has recently been shown that truncated fragments of atrophin-1, containing the expanded polyglutamine tract, accumulate in selective neuronal nuclei in a transgenic mouse model of DRPLA. Seemingly identical fragments were found in human DRPLA post-mortem brains, but not in control brains. These fragments may underlie the pathogenesis of DRPLA, and we have sought to identify the function of atrophin-1 in neuronal nuclei. Using the yeast two-hybrid system, we have found that the amino-terminus of atrophin-1 interacts with ETO - a brain-enriched protein which has recently been shown to associate with the N-CoR/mSin3A/HDAC transcriptional corepressor complex. Atrophin-1 and ETO colocalize in discrete nuclear structures when cotransfected into Neuro-2a cells. These structures stain for endogenous HDAC2 and are associated with the nuclear matrix. Furthermore, atrophin-1 and ETO cofractionate in nuclear matrix preparations from DRPLA transgenic mice. We have also found that atrophin-1 represses transcription of a reporter gene in cell transfection based assays. Taken together, these findings suggest that atrophin-1 is part of nuclear matrix-associated transcriptional corepressor complexes. We hypothesize that in DRPLA, the accumulation of truncated atrophin-1 fragments in the nuclear matrix causes neuronal dysfunction by interfering with transcriptional control mechanisms.

Program Nr: 2686 from the 1999 ASHG Annual Meeting

Evaluation of maternal genotypic effects in the etiology of nonsyndromic cleft lip and palate. *D.F. Wyszynski, T. Wu, M.H. Khoshnevisan, A.J. Miller-Chisholm, S.R. Diehl.* CEGB/NIDCR/NIH, Bethesda, MD.

Numerous investigators have searched for clues to understand the etiology of nonsyndromic cleft lip and palate (CL/P). Strong evidence of both genetic and teratogenic effects has been suggested. Recently, there has been an increasing interest in distinguishing maternal genetic effects from those due to the fetus. Animal models demonstrate that the effect of certain teratogens during pregnancy are strongly influenced by the mother's genotype. The purpose of our work was to investigate whether the maternal genotype plays an important role that can be distinguished from the fetal genotype in risk for CL/P. The transforming growth factor alpha Taq I SNP and periconceptional maternal cigarette smoking were used to test this hypothesis. Four statistical methods were used. The likelihood ratio test (LRT) distinguishes the effects of the mother's and child's genotype on risk for CL/P. The case-only and mother-only methods test for multiplicative interactions between genotype and exposure to an environmental factor. The case-only method focuses on the affected child's genotype as risk factor for CL/P. The mother-only method tests whether the mother's genotype interacts with her environmental exposure on risk for being the mother of a CL/P individual. Finally, a Fisher's exact test was performed to assess allele frequency differences between fathers and mothers of affected individuals. A total of 146 CL/P affected individuals, 90 mothers and 56 fathers were available for analysis. The case-only method provided an odds ratio (OR) = 1.43 (95% CI: 0.42-4.62), while the mother-only OR was 0.96 (0.21-4.05). The C2 SNP allele, previously shown to increase risk when occurring in the child, was less common in mothers (18%) versus fathers (30%) of CL/P affected children (p=0.158), suggesting a protective effect of this allele in the maternal genotype. This finding was also supported by an odds ratio of 0.35 (0.14-0.90) for this allele in the maternal genotype when analyzed using the LRT including multiplex sibships. These results suggest possible effects of both the maternal and child genotypes in risk of CL/P.

Program Nr: 2687 from the 1999 ASHG Annual Meeting

5,10-methylenetetrahydrofolate reductase gene polymorphism and HbA1c levels in patients with ischemic heart disease. *H. Yamagata[1], K. Kazutane[3], Y. Kanaoka[3], T. Fukuda[1], K. Obata[1], T. Miki[2], O. Yoshimura[4], I. Kondo[1]*. 1) Dept. of Hygiene, Ehime Univ. School of Med., Onsen-gun, Ehime,791-0295, Japan; 2) Dept. of Geriatric Medicine, Ehime Univ. School of Med., Ehime,791-0295, Japan; 3) Dept.of Cardiovascular Surgery, Iwakuni National Hosp., Yamaguchi, 740-8510, Japan; 4) Food and Nutrition Dept., St. Catherine Women's Junior Collage, Ehime 799-2431, Japan.

A common missense mutation (677C to T; Ala to Val) in the gene for 5,10-methylentetrahydrofolate reductase (MTHFR) is responsible for reduced enzyme activity and is associated with a modest increase in plasma homocysteine concentration. Association studies show that homozygosity for the Val allele of the MTHFR gene is an independent risk factor for atherosclerosis and thromboembolic disease. The findings, however, have not been consistent, indicative that the associations between MTHFR genotypes and diseases may be affected by additional genetic or non-genetic factors. We assessed the frequency of this common mutation in 306 patients with ischemic heart disease (IHD) and in 81 healthy controls. The frequency of the Val allele in patients with IHD did not differ significantly from that in the healthy controls (c2=1.31, df=1, P>0.25), whereas it was significantly increased in IHD patients with non-insulin-dependent diabetes mellitus (NIDDM) (0.509) as compared with IHD patients without NIDDM (0.373) (c2=6.64, df=1, P<0.010). Furthermore, patients with the Val/Val genotype were more frequent in NIDDM under poor blood-glucose control, HbA1c 7.1% groups (45.8%) than in patients with HbA1c <7.1% (13.2%); odds ratio was 7.58 (c2=12.5, df=1, P<0.0005). Our findings provide the first direct evidence that poor blood-glucose control in Val allele carriers of the MTHFR gene may be an important risk factor for IHD. cc.

Program Nr: 2688 from the 1999 ASHG Annual Meeting

Secretin gene structure, chromosome localization and mutation analysis on autism. *T. Yamagata[1], S. Aradhya[1], M. Mori[2], M.Y. Momoi[2], D.L. Nelson[1].* 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Pediatrics, Jichi Medical School, Tochigi, Japan.

Secretin is a 27-amino acid gastrointestinal hormone. The main action of secretin is to stimulate the pancreas to produce pancreatic juice; however, a role for secretin in brain has been proposed. Recently, secretin was reported to improve some symptoms of autism in some patients. Therefore, we cloned human secretin gene, identified its position in the genome and analyzed the gene for mutations in autistic patients. We designed PCR primers from the sequence of pig secretin and amplified the secretin gene from human genomic DNA. Using this PCR product as a probe, we screened the RPCI-11 BAC library and isolated the clones carrying the secretin gene. The gene is composed of four exons. On the upstream site of the secretin gene, there is a VNTR that ranges from 11 to 14 repeats of a 30 or 31 bp sequence. The secretin gene was mapped between HRAS-1 and DRD4 on 11p15.5, distal to IGF2 - H19 imprinting region first by FISH and later using specific markers in the BAC contig. The promoter region of the secretin gene is not methylated in blood cells. Twenty-five Japanese autism patients were screened by heteroduplex analysis for mutations in the secretin gene. Two patients were found to carry nucleotide substitutions. One mutation was G to C change on the E-box of the promoter and the other was C to T change in exon 3 that altered an amino acid from Pro to Leu. Each patient was heterozygous for their mutation. We screened these mutations in 58 Japanese and 50 Caucasian controls. The mutation in the promoter region was found in two Japanese and none of the Caucasian samples. It is likely to be a Japanese-specific polymorphism. The mutation in exon 3 was not found in the control samples. Family studies revealed that both the normal mother and the unaffected sister of the proband carried this mutation. Although this mutation may lead to autism in this patient, its contribution might involve reduced penetrance or possibly a sex bias. Our results suggest a possible relationship between secretin gene mutations and autism, however, further analyses are required.

Program Nr: 2689 from the 1999 ASHG Annual Meeting

A gene-wide scan for mutations in the *ALOX5AP* gene; an association study between a polymorphism in the promoter region and asthma. *C.N. Yandava*[1, 2], *A. Pillari*[1, 2], *J.M. Drazen*[1, 2]. 1) Pulmonary & Critical Care Med, Brigham & Women's Hosp, Boston, MA; 2) Harvard Medical School.

The human arachidonate 5-lipoxygenase (5-LO) activating protein (ALOX5AP), which was referred as 5-LO activating protein (FLAP) is a highly hydrophobic integral membrane protein and is localized in the nuclear envelope of certain cells. ALOX5AP binds to arachidonic acid in the presence of Ca^{2+} and present it to 5-LO. 5-LO oxidizes arachidonic acid, which results in the biosynthesis of leukotrienes. Leukotrienes are involved in a variety of biological responses including inflammation and bronchoconstriction in asthma. The organization of *ALOX5AP* gene is known and it consists 5 small exons. In order to find association between asthma and *ALOX5AP*, we scanned all 5 exons, 5' UTR and 3' UTR regions for mutations by single strand conformation polymorphism analysis (SSCP). Variations were observed in the promoter region of the gene. The amplified PCR products of common and variant samples were sequenced directly on an automated ABI prism 377 sequencer. The observed change corresponds to a mutation form G to A at -337 nucleotide position from the translation initiation site (G-377A). This change results in the loss of the restriction site *Bss*SI. This base change is close to an AP-2 site in the promoter. 200 Caucasian controls and 150 asthmatic patients were genotyped for this mutation by restriction fragment length polymorphism analysis (RFLA). The allele frequencies for the common allele are 0.96 and 0.96 in the controls and asthmatics respectively. There are no differences between normal and asthmatic Caucasian populations.

Program Nr: 2690 from the 1999 ASHG Annual Meeting

Protective effect of CCR5 32 base pairs deletion in Crohn's Disease. *H. Zouali[1,2], M. Chamaillard[1,2], JF. Colombel & EPIMAD[2], J. Belaiche & the GETAID[2], JP. Cezard & the EPGWGPIBD[2], C. Tysk[2], S. Almer[2], M. Gasull[2], V. Binder[2], S. Lesage[1,2], J. Macry[2], G. Thomas[1,2], JP. Hugot[1,2]*. 1) Fondation Jean Dausset, Paris, France; 2) European Concerted Action on the Genetics of Inflammatory Bowel Diseases.

Background : During the inflammation in Inflammatory Bowel Diseases (IBD), the chemokines are increased in the intestinal tissue. The effect of chemokines is mediated by a number of receptors including CCR5. The CCR5 gene is located on chromosome 3 in the genetic region suspected to contain a gene involved in IBD susceptibility. The CCR5 32 base pair deletion (CCR5-D32) which generates a premature protein, has been recently associated with IBD.

Aim of the study : To determine whether CCR5-D32 might form a susceptibility allele for IBD.

Patients and methods : A total of 395 IBD families with at least one affected child were genotyped for CCR5-D32. Out of these families, 247 had only Crohn's Disease (CD) patients, 56 were pure Ulcerative Colitis disease (UC) patients, and 92 mixed families had CD's and UC's patients. The DNA samples of IBD family members were typed by PCR followed by electrophoresis on agarose gel. Genotyping data were analyzed using a transmission disequilibrium test (TDT-like from the Analyse package).

Results : The CCR5-D32 allele was less often transmitted to CD's patients than expected by chance (44 transmitted alleles versus 66 untransmitted ; p = 0.036). No transmission disequilibrium was observed for UC's patients (18 versus 19 ; p = 0,869) or healthy relatives (59 versus 61 ; p = 0,855).

Conclusion : The defective CCR5D32 allele may have a protective effect for CD. Similar results have been reported in both patients infected with HIV and in patients with Rheumatoid Arthritis. Since the RANTES chemokine is overexpressed in CD granulomas, we can postulate that the normal expression of chemokines and their receptors may be necessary to develop an inflammation in IBD patients.

Program Nr: 2691 from the 1999 ASHG Annual Meeting

Etiologic diversity for deafness on the island of Providencia - Colombia. *M.C. Lattig[1], R. Morell[4], T.B. Friedman[4], G. Tamayo[2], J.I. Uribe[3], J.E. Bernal[1], S.L. Plaza[1], M.L. Tamayo[1]*. 1) Instituto de Genetica Humana, Universidad Javeriana. Bogota, Colombia; 2) Fundacion Oftalmologica Nacional, Bogota, Colombia; 3) Hospital San Ignacio - Universidad Javeriana. Bogota, Colombia; 4) National Institute on Deafness and Other Communication Disorders (NIDCD), NIH, Rockville, MD. USA.

Providencia is an island located in the Caribbean Ocean, northwest of Colombia. The island's population is diverse, consisting of individuals from Holland, England, France, Spain and Africans. An unusually high frequency of hearing loss was found on this island (5.0 in 1,000 individuals). Non-syndromic autosomal recessive deafness accounts for 47% (8/17) of the deaf population of Providencia. Waardenburg Syndrome accounts for 29% of the hearing impaired (5/17) with the remaining 24% (4/17) isolated cases of deafness. The 30delG mutation of GJB2, (connexin 26) is responsible for the deafness observed in the 8 individuals with autosomal recessive non-syndromic hearing loss. The isolated individuals with nonsyndromic deafness do not have this mutation. The five cases of deafness associated with Waardenburg Syndrome (WS) show clear autosomal dominant inheritance. They did not carry the 30delG mutation and also appear not to have a mutation in either PAX3 or MITF. The present study reports an isolated and endogamous community with at least two etiologies for deafness.

Program Nr: 2692 from the 1999 ASHG Annual Meeting

PROP1 gene mutation analysis in patients with idiopathic hypogonadotropic hypogonadism. *L.C. Layman[1], J.D. Cogan[2], J. Carneal[2], K.L. Layman[1], D.V. Yu[1], S. Kale[1], J.A. Phillips III[2].* 1) Section of Reproductive Endocrinology & Infertility, Dept OB/GYN, Univ Chicago, Chicago, IL; 2) Division of Genetics, Vanderbilt University, Nashville, TN.

PROP1 gene mutations have been identified in patients with combined pituitary deficiency, who present with short stature, hypothyroidism, and delayed puberty. These patients have deficient levels of growth hormone, thyroid stimulating hormone (TSH), prolactin, luteinizing hormone (LH), and follicle stimulating hormone (FSH). Patients with idiopathic hypogonadotropic hypogonadism (IHH) have delayed puberty with low serum gonadotropin levels, but normal growth hormone, TSH, and prolactin levels. We hypothesized that some PROP1 gene mutations might only result in gonadotropin deficiency, sparing other pituitary hormones. Fifty IHH patients and 30 controls were studied using gene screening techniques. DNA from each patient was amplified by the polymerase chain reaction (PCR) for each of the three exons and splice junctions of the PROP1 gene. PCR products were then subjected to dideoxyfingerprinting and/or denaturing gradient gel electrophoresis (using GC-clamped fragments). Several variant fragments were identified in the IHH patients, but not in controls. Two IHH patients were heterozygous for a silent Pro to Pro change (exon 2 G174A) not seen in any of the 30 controls, and one IHH patient was heterozygous for a 1 bp deletion within intron 1 (IVS 1 delG1031). Although no definitive PROP1 gene mutations have been identified to date, additional variant fragments are currently being characterized. Even if some variants are ultimately demonstrated to represent mutations, PROP1 gene mutations are an uncommon cause of isolated IHH in humans.

Program Nr: 2693 from the 1999 ASHG Annual Meeting

Fragile X syndrome carrier females with full mutations inherited from a normal transmitting male. *L.P. Lazarou[1], S. Warburton[2], C.E. Roberts[1], V.H. Lindley[2], S. Bartlett[1], H.E. Hughes[1], L. Meredith[1], D. Ravine[1]*. 1) Institute of Medical Genetics, University Hospital of Wales, Cardiff, United Kingdom; 2) West Midland Regional Genetics Service, Birmingham,UK.

We report findings in a family having males affected with Fragile X syndrome and a normal transmitting male who has five phenotypically normal daughters, four with one partner and one with another. Two daughters have been shown to have a premutation sized mutations. Two other daughters have fully methylated expansions that were classified as full mutations. One of these daughters with a full mutation smear associated with cytogenetically visible fragile sites in 12% of cells has a Fragile X syndrome affected son. The other with fragile sites in 22% of cells has a fully methylated expansion of approximately 196 repeats. The mother of the full mutation carrying females, tested normal at the FMR1 gene locus. This observation indicates that normal transmitting males for Fragile X syndrome can on rare occasions have offspring with fully methylated expanded alleles.

Program Nr: 2694 from the 1999 ASHG Annual Meeting

Sarcospan-deficient mice maintain sarcolemmal expression of other components of the dystrophin-glycoprotein complex. *C.S. Lebakken[1], D.P. Venzke[1], R.F. Hrstka[2], J.A. Faulkner[3], R.A. Williamson[2], K.P. Campbell[1]*. 1) Departments of Physiology & Biophysics and Neurology, HHMI, University of Iowa College of Medicine, Iowa City, IA; 2) Department of Obstetrics and Gynecology, University of Iowa College of Medicine, Iowa City, IA; 3) Institute of Gerontology, University of Michigan, Ann Arbor, MI.

Sarcospan is a 25-kDa integral membrane component of the dystrophin-glycoprotein complex (DGC). It is unique among the components of the DGC in that it contains four transmembrane domains and intracellular C- and N-terminal domains. Proteins with such topology are known to facilitate protein-protein interactions at the plasma membrane. Sarcospan has recently been shown to be a component of the sarcoglycan sub-complex within both the skeletal and smooth muscle DGC. Stable expression of sarcospan at the sarcolemma is exquisitely dependent upon expression of the sarcoglycan sub-complex as has been shown in mice deficient for a-sarcoglycan and in the BIO 14.6 hamster, animal models for limb-girdle muscular dystrophies. To explore the function of sarcospan we have characterized its expression in embryonic and adult mouse tissues and have developed sarcospan-deficient mice by disruption of the murine sarcospan gene through targeted homologous recombination. Northern blot analysis indicates that sarcospan transcript is first detected between embryonic days 11 and 15, likely coinciding with secondary myogenesis. In embryonic and adult murine tissue, sarcospan protein expression is limited to skeletal, cardiac, and smooth muscle tissues. Surprisingly, sarcospan-deficient mice maintain expression of the other components of the dystrophin-glycoprotein complex at the sarcolemma including the sarcoglycans and a- and b-dystroglycan. No gross histological abnormalities of muscle from the mice have been found, and serum creatine kinase levels are not elevated. Detailed biochemical analysis of the DGC from these mice is currently being perfomed, as well as physiological tests to study their muscle function. To date, there have been no human mutations found within sarcospan that cause a muscular dystrophy which is consistent with this phenotype.

Program Nr: 2695 from the 1999 ASHG Annual Meeting

***NPHS1* Mutations in Non-Finnish CNF Populations.** *H.J. Lee, O. Gribouval, C. Antignac.* INSERM U423, Hopital Necker, PARIS, France.

Congenital nephrotic syndrome of the Finnish type (CNF) is an autosomal recessive disorder caused by mutations in the nephrin gene, *NPHS1*. *NPHS1* consists of 29 exons spanning 26kb of the chromosomal region 19q13.1. Although CNF exists predominately in Finland as a part of the Finnish disease heritage, many cases have been observed elsewhere. Linkage studies of the CNF locus in non-Finnish families correlate with patterns of disease inheritance, and a number of mutations have already been characterized in individuals of non-Finnish origins (*Am. J. Hum. Genet.* 64:51-61, 1999).

In this study, we screened 15 patients of European and North African origins for mutations in all 29 exons of NPHS1 by single strand conformation polymorphism analysis (SSCP) and direct sequencing. We detected a total of 16 mutations in 11 of the patients screened, 6 of the families being consanguineous. Seven of these mutations have been previously described, and 8 are novel. Of the novel mutations, 4 are micro-deletions. Three of these deletions cause frameshifts, leading to truncated proteins. The 4 point mutations lead to the following single amino acid substitutions: R379W, S398P, R460E, P519S. The first of these mutations was found in two patients.

Furthermore, we searched in a GA repeat sequence of the putative promoter region for a (GA) deletion previously suspected of being responsible for the disease (*Am. J. Hum. Genet.* 64:51-61, 1999). A number of $(GA)_n$ deletions (n=1 or 2) were found in a population of healthy individuals unassociated with CNF. The allele frequency (0.04) is far above that which would be expected if a deletion of one or more GA pairs indeed leads to the disease. Therefore, the difference in length of the GA repeat seems to be a polymorphism. We are in the process of screening 18 new non-Finnish patients for mutations in *NPHS1*, permitting additional prenatal diagnoses and the verification of the absence of any genetic heterogeneity. Further characterization of mutations may also allow the identification of functionally important domains of nephrin.

Program Nr: 2696 from the 1999 ASHG Annual Meeting

Novel and prevalent mutations of the LDL receptor gene in Korean patients with familial hypercholesterolemia.

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Familial hypercholesterolemia (FH) is an autosomal dominant genetic disorder caused by mutations in the LDL receptor gene. Although approximately 300 different mutations of the LDL receptor gene have been identified in the FH patients of various races, molecular defects in the Korean patients with FH have little been characterized. To examine kinds and prevalence of point mutations of the LDL receptor gene in the Korean FH patients, we have amplified each of the 18 exons including adjacent intron sequences of the LDL receptor gene by PCR and screened for the presence of mutations by SSCP and heteroduplex analyses. The specific mutations were identified by DNA sequencing of the aberrant exons. Three substitution mutations (E119K, E207K, and P664L), a novel deletion mutation (DM510-I521), a novel insertion mutation (D548insCTAG), and a splicing junction mutation (IVS13-1G®A) were found in the 10 Korean FH patients. Some of these mutations were recurrent in the unrelated patients; DM510-I521 (3 cases), E207K (2 cases), and P664L (2 cases). By RT-PCR of the fibroblast mRNA from the patient with the novel D548insCTAG mutation in which 4 nucleotides, CTAG, were duplicated at the 3' end of the exon 11, we found that the transcript from the mutant locus was not properly spliced and thus would lead to a non-functional truncated polypeptide. This study demonstrates the presence of the novel and prevalent mutations of the LDL receptor gene in the Korean FH patients and also makes it possible to do prognosis and prenatal diagnosis in Korean families at high risk of FH. [Supported by MOST of Korea (MM-1-1)].

Program Nr: 2697 from the 1999 ASHG Annual Meeting

Pseudoxanthoma elasticum maps to an 820 kb region of the p13.1 region of chromosome 16. *O. Le Saux[1], Z. Urban[1], H.H.H. Göring[2], K. Csiszar[1], F.M. Pope[3], A. Richard[3], I. Pasquali-Ronquetti[4], L. Bercovitch[4], M.G. Lebwohl[4], M. Breuning[5], P. van der Berg[5], L. Kornet[5], N. Doggett[4], J. Ott[2], P.T.V.M. Paulus[5], A.A.B. Bergen[5], C.D. Boyd[1]*. 1) Pacific. Biomedical. Research. Center, University of Hawaii, Honolulu, HI; 2) Department of genetics and Development, Columbia University, New York, NY; 3) Institute of Medical genetic, University Hospital of Wales, Cardiff, The United Kingdom; 4) The PXE Consortium and PXE International Inc., Sharon MA; 5) The Netherlands Ophthalmic Research Institute, Amsterdam, The Netherlands.

We have performed linkage analysis on 21 families with Pseudoxanthoma elasticum (PXE) using 10 polymorphic markers located on chromosome 16p13.1. The gene(s) responsible for the PXE phenotype was localized to an 8 cM region of 16p13.1 between markers D16S500 and D16S3041 with a maximum lod score of 8.1 at a recombination fraction of 0.04 for marker D16S3017. The lack of any locus heterogeneity suggests that the major predisposing allele is located in this region. Haplotype studies of a total of 36 PXE families identified several recombinations that further confined the PXE gene(s) to a region between markers D16S3060 and D16S79. This PXE locus was identified within a single YAC clone and several overlapping BAC recombinants. From sequence analysis of the BAC recombinants, it is clear that the distance between markers D16S3060 and D16S79 is about 820 kb and contains a total of 10 genes including 4 pseudogenes. We predict that mutations in one (or more) of the expressed genes in the locus will be responsible for the PXE phenotype in these families.

Program Nr: 2698 from the 1999 ASHG Annual Meeting

Production and characterization of tau transgenic mice. *J. Lewis[1], N. Mehta[1], E. McGowan[1], P. Davies[2], M. Hutton[1]*. 1) Neurogenetics, Mayo Clinc, Jacksonville, FL; 2) Pathology, Albert Einstein College of Medicine, Bronx, NY.

The microtubule associated protein tau, which promotes microtubule assemble and stabilization, has been implicated in various neurodegenerative diseases such as frontotemporal dementia and Alzheimer's disease. Six different isoforms of tau are produced by alternative splicing of exons 2, 3, and 10. In order to explore the role of wild-type and mutant tau isoforms in the neurodegenerative process, we have created multiple lines of tau transgenic mice expressing one of four different tau cDNAs (wild-type 3 repeat, wild-type 4 repeat, V337M 3 repeat, and P301L 4 repeat). The tau cDNAs driven by the mouse prion promoter (moPrP) were individually injected into fertilized eggs (C57BL/DBA2/SW) to yield founder lines. Western analysis using a human exon 1 (E1) specific antibody demonstrated ubiquitous human tau expression in the forebrains of tau transgenic mice. Based on Western analysis with an antibody that recognized human and murine tau isoforms, we have demonstrated a range of expression from endogenous to 10 fold overexpression of human tau when compared to murine tau. In situ hybridization showed the highest levels of expression in the hippocampus, cortex, striatum, and the thalamus. Aging studies of the tau mice as well as tau mice bred to the APP transgenic (Tg2576) mice are currently underway.

Program Nr: 2699 from the 1999 ASHG Annual Meeting

Mutation analysis of the human muscle LIM protein in patients of familial dilated cardiomyopathy. *D. Li, O. Gonzalez, M.A. Quinones, W.A. Zoghbi, R. Hill, L.L. Bachinski, R. Roberts.* Dept. of Medicine/cardiology, Baylor College of Medicine, Houston, TX.

Dilated cardiomyopathy (DCM) is the most common form of primary myocardial disease. Clinically, it is characterized by ventricular dilatation, systolic dysfunction, sudden death, and heart failure. About 50% of DCM is idiopathic, of which at least 20% is familial. Progress in unraveling the genetic causes of idiopathic DCM has been slow compared to that of hypertrophic cardiomyopathy. Using the candidate gene approach, the mutations of cardiac actin and desmin were shown to be responsible for a small portion of familial cases of idiopathic DCM. These emerging evidences support the hypothesis that DCM is a disease of myocardial force transmission, mainly caused by molecular disturbance of cytoskeletal proteins. The striated muscle-specific LIM -only protein (MLP) is a positive regulator of myogenic differentiation. It is highly expressed in the cardiac myocytes during development and in the adult. Recent evidence suggest that MLP may act as a scaffold protein to promote protein assembly along the actin-based cytoskeleton. Interestingly, MLP-knockout mice exhibited the structural and functional abnormality very similar to dilated cardiomyopathy in humans. To determine whether a molecular defect of MLP induces dilated cardiomyopathy in humans, 43 DCM index cases underwent clinical evaluation and DNA analysis. Diagnostic criteria, detected by echocardiography, consisted of ventricular dimension of >2.7 cm/m2 with an ejection fraction <50% in the absence of other potential causes. The exons of the MLP gene were amplified from the genomic DNA of patients by PCR ,and were sequenced in both sense and antisense directions on ABI 310 genetic analyzer. No mutation was found in this group of patients while a previously non-reported silent G/A sequence variation was detected in exon 3 at nucleotide 381 (U72899). Sequence of eighty normal control individuals did not show difference in the frequence of this sequence variation when compare with the DCM patients. Thus, we suggest that MLP defects, if they occur, are not a common cause of familial DCM.

Program Nr: 2700 from the 1999 ASHG Annual Meeting

Intranuclear huntingtin with expanded polyglutamine alters gene expression and cellular function in PC12 cells.
S-H. Li, A.L. Cheng, H. Li, X-J. Li. Department of Genetics, Emory University, Atlanta, GA.

Expanded polyglutamine tracts cause huntingtin and other proteins to accumulate and aggregate in neuronal nuclei. Whether the intranuclear aggregation or localization of a polyglutamine protein initiates cellular pathology remains controversial. We established stably transfected PC12 cells that express the N-terminal fragment of huntingtin containing 20 (20Q) or 150 (150Q) glutamine residues. The 150Q protein is predominantly present in the nuclei, whereas the 20Q protein is distributed throughout the cytoplasm. Electron microscopic examination confirmed that most of the 150Q protein is diffuse in the nucleus with very few microscopic aggregates observed. Compared to parental PC12 cells and cells expressing 20Q, cells expressing 150Q display abnormal morphology, lack normal neurite development, die more rapidly, and are more susceptible to apoptotic stimulation. The extent of these cellular defects in 150Q cells is correlated with the expression level of the 150Q protein. Differential display PCR and expression studies show that cells expressing 150Q have altered expression of multiple genes, including those that are important for neurite outgrowth. Our study suggests that mutant huntingtin in the nucleus is able to induce multiple cellular defects by interfering with gene expression even in the absence of aggregation.

Program Nr: 2701 from the 1999 ASHG Annual Meeting

The role of MSX1 in the Etiology of Congenital Hypdontia. *A.C. Lidral, B. Reising.* Sect Orthodontics, Postle Hall, Ohio State Univ, Columbus, OH.

The homeobox containing genes play a critical role in cell differentiation during the earliest stages of embryogenesis. Expression of the non-clustered homeobox gene MSX1 has been strongly implicated as vital to the normal development of various craniofacial structures. Msx1 homozygous deficient mice display cleft palate, disruption of dental and alveolar development and other craniofacial abnormalities. Previously, an Arg31Pro missense homeodomain mutation in a family with autosomal dominant agenesis of second premolars and third molars has been described. Congenital hypodontia occurs in 2-10% of the population, excluding missing third molars. The biology of dental development involves reciprical epithelial-mesynchymal interactions and thus serves as a model for the development of other organs which also have these interactions. We hypothesize that MSX1 mutations are present in some instances of congenital hypodontia of the permanent human dentition. To test this hypothesis, DNA samples were obtained from the buccal mucosa cells of 92 individuals (82 nuclear families) demonstrating congenital agenesis of at least one permanent tooth with the exception of third molars. A mutation screen of the MSX1 coding region was performed by SSCA of overlapping amplified segments. In this study population only 9% had hypodontia involving 5 or more teeth, while the majority (77%) had hypodontia of only 1 or 2 teeth. A T620A mutation resulting in a Met61Lys substitution, which is in the N-terminal region upstream from of the homeobox, was identified in two siblings from a family with 16 members affected with severe hypodontia segregating in an autosomal dominant manner. This mutation was not found in 80 normal chromosomes. This new mutation, which occurs in a region involved in protein-protein interactions, confirms that MSX1 mutations result in severe congenital hypodontia. The lack of mutations in individuals missing only 1 or 2 teeth suggests that MSX1 is not involved in the etiology of simple hypodontia, but rather it is important for the development of multiple teeth. In addition, the teeth that did develop in these individuals were generally normal, suggesting that MSX1 does not have a major role in determining tooth size and morphology.

Program Nr: 2702 from the 1999 ASHG Annual Meeting

Deletion of Col2A1 IVS7 5donor splice site in Stickler syndrome with SEMD: An expansion of Type II Collagenopathy phenotypic spectrum. *T. Lin[1], F.L. Lacbawan[2], J. Davis[2], M. Muenke[2], L.C. Wong[1], C.A. Francomano[2]*. 1) Inst Molec/Human Genetics, Georgetown Univ Medical Ctr, Washington, DC; 2) National Human Genome Research Institute, NIH, Bethesda MD.

Type II collagenopathy presents in a spectrum of chondrodysplasias with variable severity. In decreasing severity it includes achondrogenesis type 2, hypochondrogenesis, spondyloepimetaphyseal congenita, spondyloepimetaphyseal (SEMD), Kniest dysplasia, and Stickler syndrome. Col2A1 mutations in Stickler syndrome patients are predominantly missense, nonsense and frameshift mutations. However, in the original Stickler syndrome kindred, A to G transition at the -2 3' acceptor splice site of IVS17 eliminates 16bp because a cryptic splice site is utilized. Except for the missense mutation, these changes result in premature termination of translation.

We describe here a 6 yo boy with midface hypoplasia, high myopia, retinal detachment, bilateral SNHL, micrognathia, cleft palate, hyperextensible joints, and joint pains consistent with Stickler syndrome. In addition, he had platyspondyly, abnormal acetabulum, epiphyseal hypoplasia, remarkable metaphyseal flaring with decreased US/LS ratio, prominent clavicle, pectus carinatum, flexion contractures, decreased cervical mobility and kyphosis, and mild developmental delay. With TTGE and sequencing, we found a 4 bp deletion (GTAA) at the +1®+4 5' donor splice site of Col2A1 IVS7 in his leukocyte genomic DNA. The deletion brings a GTAT consensus sequence at the splice junction. Conceivably, this may eventuate in a splicing error or exon skipping. RT-PCR of polyA+ RNA is being done to demonstrate the outcome of the deletion.

The overlapping clinical features of SEMD and Stickler syndrome in this patient extends the phenotypic spectrum of Type II Collagenopathy.

Program Nr: 2703 from the 1999 ASHG Annual Meeting

HNBCoi: A novel Na^+/HCO_3^- cotransporter in the 5q region linked to ovarian dysgenesis and sensorineural deafness (Perrault Syndrome). *L. Lipovich[1], J.M. Opitz[3], J. Morrow[2], M.K. Lee[2], R. Person[2], M.-C. King[2], E.D. Lynch[2]*. 1) Dept. of Molecular Biotechnology, Univ. of Washington, Seattle, WA; 2) Div. of Medical Genetics, Univ. of Washington, Seattle, WA; 3) Primary Children's Medical Center, Salt Lake City, UT.

Perrault Syndrome is characterized primarily by sensorineural autosomal recessive deafness in both males and females, which occurs along with ovarian dysgenesis in the deaf females (Pallister PD, Opitz JM; Am J Med Genet 1979; 4:239-46). Genome-wide linkage analysis of a single large family with the ABI Prism Linkage Mapping Set, Version 2, revealed a region of likely linkage along 5q. The analysis excluded DFNA1 and DFNA15 as candidate loci. Linkage in this region was also found in two smaller, unrelated Perrault Syndrome families. The combined LOD score from these three families was +3.57 at seven markers spanning approximately 3 cM. A search for known genes, transcript identification, and exon prediction were conducted on all publicly available sequences from the region using SeqHelp (Lee MK, et al; Genome Res 1998; 8:306-12). This analysis indicated the presence of a novel Na^+/HCO_3^- cotransporter in the region, which we termed HNBCoi. Due to the relevance of ion channel mutations to inherited deafness, HNBCoi is currently being analyzed as a Perrault Syndrome candidate gene. SeqHelp analysis and sequencing of IMAGE clones corresponding to HNBCoi enabled us to identify 1036 nt of contiguous cDNA sequence, partially determine the genomic structure of HNBCoi, and sequence portions of the gene in the three affected families. To localize the 5' end of the gene, RACE and cDNA library screening are being conducted. LD-PCR has been used to amplify previously unknown genomic sequence containing the 3' end of the gene. Northern blot analysis yielded 4.3-kb and 6.0-kb transcripts at high levels in the kidney, with no expression in the heart, brain, placenta, lung, liver, skeletal muscle, or pancreas. We successfully detected an HNBCoi transcript in ovarian mRNA by RT-PCR, with sequence identical to that of the IMAGE clones. Ovarian expression of HNBCoi is interesting in the light of ovarian dysgenesis in Perrault Syndrome.

Program Nr: 2704 from the 1999 ASHG Annual Meeting

Aminoterminal end of the single CLD gene transcript is posttranslationally cleaved both in normal and inflammatory colon epithelium. *H. Lohi[1], S. Haila[1], P. Hoglund[1], P. Puolakkainen[2], J. Kere[3].* 1) Dept. of Medical Genetics, Haartman Institute, Helsinki, Finland; 2) Dept. of Surgery, Helsinki Univ. Central Hospital, Helsinki, Finland; 3) Finnish Genome Center, University of Helsinki, Helsinki, Finland.

Congenital chloride diarrhea (CLD) is a recessively inherited disorder of intestinal electrolyte absorption involving Cl-/HCO3- exchange. In CLD patients, watery stools containing an excess of chloride begin before birth, leading to polyhydramnios and often premature birth. Neonates develop dehydration and electrolyte disturbancies. The CLD gene encodes a Na+ independent anion exchanger expressed mainly in the apical surface of the colonic epithelium. We have studied the expression and processing of the CLD protein and mRNA in normal and inflammatory colon tissues. We produced rabbit polyclonal antibodies against different regions of the CLD protein. We found that about 10 kDa, corresponding to approximately the first two coding exons of the aminoterminal end of the CLD protein is posttranslationally cleaved, leading to the functional size of the protein of about 75 kDa. The aminoterminal antibody recognizes only the larger about 85 kDa proform of the CLD protein both in normal and inflammatory colon epithelium in Western analysis. Carboxyterminal antibodies recognize both the pro- and functional forms of the protein, suggestive for the aminoterminal cleavage. Futhermore, a single transcript for the gene in Northern analysis and in 5 RACE experiments suggest posttranslational cleavage of the protein rather than differentially spliced forms of the gene. There are no differences in the expression or processing of the CLD protein in inflammatory colon cells compared to normal colon. So far we have found three disease-causing point mutations in addition to three frameshift mutations in the aminoterminal region of the CLD gene. The three inframe mutations suggest that the aminoterminus is necessary for the proper processing or function of the protein. Ongoing studies try to resolve the functional roles of the amino- and carboxyterminus and effects of the mutations for processing and targeting in different cell lines.

Program Nr: 2705 from the 1999 ASHG Annual Meeting

A physical and transcriptional map of the SPG9 locus on 10q23.3-q24.2. *C. Lo Nigro[1], R. Cusano[1], M. Scaranari[1], V. Brescia Morra[2], G. De Michele[2], R. Ravazzolo[1,3], M. Seri[1].* 1) Lab. Molecular Genetics, Gaslini Institute, Genoa, Italy; 2) Dept. Neurological Science, Federico II University, Naples, Italy; 3) Dept. Oncology, Biology and Genetics, University of Genoa, Italy.

We have recently identified the SPG9 locus (OMIM:601162) on chromosome 10q23.3-q24.2, in a 12 cM interval between markers D10S564-D10S603. Using a genomewide approach in a large Italian family, we mapped this rare form of autosomal dominant spastic paraparesis complicated by amyotrophy, bilateral cataracts, gastroesophageal reflux with persistent vomiting. In order to identify the gene responsible for the disease, we constructed a contig of 19 YACs and we positioned 16 markers, which were used in the linkage analysis performed on the original pedigree. Based on these physical data, the estimated size of the critical region is about 6-7 Mb. GeneMap 99 reports 173 ESTs in the D10S564-D10S603 interval, which could represent candidate genes because of their location and function or expression. A bioinformatic approach, based on BLASTN and BLASTX, allowed us to cluster those ESTs belonging to the same transcript and to reduce to 118 the number of single transcripts. We are constructing a transcriptional map of the SPG9 locus, positioning by PCR the ESTs on the YACs contig. Firstly, we are selecting transcripts which: - are specifically expressed in brain; - contain a trinucleotide repeat, as suggested by the presence of the anticipation phenomenon in the original pedigree; - encode for mitochondrial proteins, since recently a nuclear-encoded mitochondrial metalloprotease has been demonstrated to be involved in a recessive form of spastic paraplegia in 16q24.3. In particular, we positioned within the critical region the COX15 gene, which is a constituent of the inner mitochondrial membrane and may be involved in the assembly of the Cytochrome-c oxidase (COX) protein backbone. COX15 cDNA analysis on RNA extracted from cultured fibroblast cells of an affected member of the original family is in progress. Histologic, histoenzymatic and ultrastructural studies of a muscle biopsy sample from the same patient is going on to test the possible mitochondrial involvement in the pathogenesis of the disease.

Program Nr: 2706 from the 1999 ASHG Annual Meeting

A candidate gene approach in Xcen-Xq21 toward the cloning of the FG syndrome gene (FGS1). *A.M. Lossi[1], L. Villard[1], L. Colleaux[1], S. Briault[2], C. Moraine[2], C. Schwartz[3], M. Fontes[1].* 1) INSERM U491, Faculté de Médecine La Timone, 27 Bd. Jean Moulin 13385 Marseille, France; 2) Dpt. de Génétique, INSERM U316, CHU Bretonneau, 37044 Tours, France; 3) Greenwood Genetic Center, Greenwood SC, USA.

FG syndrome (MIM 305450) is an X-linked mental retardation syndrome characterized by severe mental retardation, macrocephaly, facial dysmorphism and anomalies of the development of the distal part of the digestive tract, often resulting in anteposed or imperforated anus. Linkage studies have demonstrated that a gene (FGS1) responsible for the disease in 80% of the FG syndrome families is located in the Xq12-Xq21 region of the human X chromosome (with a Zmax=3.39 at theta=0 for DXS441 in a study based on 10 families). In a first step, we tested the potential involvment of the XNP gene which is located in the linkage interval (and close to the marker giving the maximal value of Z) and is responsible for a mental retardation syndrome associated with developmental anomalies (ATR-X and Juberg-Marsidi syndromes). We have analyzed the XNP genomic region and transcript from two FG patients, members of families linked to the region, and have found no abnormality. This result probably rules out the involvment of the XNP gene in the etiology of the FG syndrome. Since the available families are small, the linkage interval in which the causative gene is located remains large. This is the reason why we have undertaken a systematic candidate gene approach in this region of the human X chromosome to identify the FG syndrome gene. In a second step, we are thus currently using a detailed transcriptional map of Xcen-Xq21 to apply a systematic candidate gene approach. Data will be presented based on the analysis of ten different candidate genes.

Program Nr: 2707 from the 1999 ASHG Annual Meeting

ISOLATION OF THE PROMOTER REGION OF THE WD GENE AND CHARACTERIZATION OF THE MOST FREQUENT MUTATION IN THE SARDINIAN POPULATION. *G. Loudianos[1,2], V. Dessi[2], M. Lovicu[3], A. Angius[4], A. Figus[5], F. Lilliu[1,2], S. De Virgiliis[2], V. Deidda[2], P. Moi[1,2], M. Pirastu[4], A. Cao[1,2,3]*. 1) Ospedale Regionale per Le Microcitemie, ASL 8, Cagliari; 2) ICBEE- Università di Cagliari; 3) IRTAM CNR-Cagliari; 4) IGM CNR-Alghero; 5) IMI, Università di Cagliari.

As reported in our previous studies, despite an extensive characterization of the coding and splicing regions of the Wilson disease (WD) gene we had before failed to define the disease-causing mutation of the most common haplotype in the Sardinian population, which accounts for 60.5 % of the WD chromosomes. In order to extend our mutation analysis to the 5' UTR and to the promoter region, we have obtained and analysed 1,228 bp of novel 5 sequences. This DNA region was predicted by NIX analysis to contain two promoters oriented in a divergent, head to head fashion. The promoter immediately 5 to the WD gene contained a GC-rich island, a CAAT and a TATA consensus sequences as well as potential binding sites for transcription factors and metal responsive elements, all features suggestive of an housekeeping and metal regulated gene as it was expected for the WD gene. Comparative sequence analysis of this putative WD promoter region has led to the discovery of a 15nt deletion at -427 bp from the translation start site in all the 92 Sardinian chromosomes carrying the most common WD haplotype. Functional studies with a luciferase reporter gene demonstrated a 75% reduction in the transcriptional activity of the mutated promoter sequence compared to the normal control. These data clearly indicate that the fragment we have analysed contains the whole WD promoter and that the 15nt deletion is the disease-causing mutation associated with the common Sardinian WD haplotype. The addition of this novel common mutation brings the rate of the characterized WD mutations in Sardinian chromosomes to 92%. Thus the high frequency of the WD in Sardinians and the almost complete coverage of the mutations by DNA analysis make now feasible and convenient a newborn screening program that should allow early diagnosis and prevention of the Wilson disease progression at a preclinical stage.

Program Nr: 2708 from the 1999 ASHG Annual Meeting

Toward the elucidation of the molecular mechanism of the genomic recombination leading to duplication/deletion rearrangement associated with Pelizaeus-Merzbacher disease. *J.R. Lupski[1], J. Takanashi[2], K. Murayama[3], T.D. Bird[4], K. Inoue[1]*. 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Pediatrics, Chiba University, Chiba, Japan; 3) Pediatrics, National Rehabilitation Center for Disabled Children, Tokyo, Japan; 4) Neurology, University of Washington, Seatle, WA.

Duplication of the genomic region containing the *PLP*gene is the major cause of the CNS dymyelinaing disorder, Pelizaeus-Merzbacher disease (PMD). Rarely, deletion of the *PLP*gene is also responsible for PMD. We investigated the hypothesis that unequal sister chromatic exchange in male meiosis is likely to be the common mechanism for the recombination of the duplicated genomic region, even though the breakpoints of the duplications are strikingly variable. Here we show further findings to clarify the molecular mechanism of the *PLP*dulication/deletion. In two families with different size duplications, we performed interphase FISH and haplotype analyses on maternal grandparents to determine that the duplications actually occurred in the male meiosis by the sister chromatic exchange. In one family with *PLP*deletion, submicroscopic interstitial translocation of the Xq22 to 19qter was identified in the patient's mother, which resulted in the *PLP*deletion in the patient. Sequencing analysis of the breakpoint showed that *Alu-Alu* recombination is the mechanism. Breakpoints of another *PLP*deletion family have been mapped in a different region with those of the *PLP*duplications, suggesting different genomic mechanism between duplication and deletion. These data indicate complex genomic structure and mechanism of the rearrangement leading to duplication/deletion of the *PLP*gene.

Program Nr: 2709 from the 1999 ASHG Annual Meeting

A splicing mutation in dysferlin produces limb-girdle muscular dystrophy with inflammation. *C.T Ly[1], H. Rosenmann[2], S. Rosenbaum[3], D. Soffer[2], W. Jiang[1], L.V.B. Anderson[4], Z. Argov[2], E.M. McNally[1]*. 1) Medicine, Univ of Chicago, Chicago, IL; 2) Neurology, Hadassah University Hospital; 3) Mol Biol and Genetic Engineering, Hadassah University Hospital, Jerusalem, Israel; 4) Neurology, Univ. of Newcastle, Newcastle on Tyne.

Mutations in dysferlin were recently described in patients with both the mild Miyoshi Myopathy and the more severe Limb-Girdle Muscular Dystrophy (LGMD) type 2B (Bashir et al., Liu et al., 1998). Despite striking phenotypic differences, the types of dysferlin mutations associated with Miyoshi Myopathy and LGMD type 2B do not differ significantly. To extend the phenotype associated with dysferlin mutations, we have identified a large inbred pedigree of Yemenite Jewish descent that exhibited limb-girdle muscular dystrophy. The phenotype in these patients includes progressive, proximal muscular weakness with markedly elevated serum creatine kinase levels. In contrast to Duchenne Muscular Dystrophy and sarcoglycan-mediated LGMDs, these LGMD 2B patients have normal development and muscle strength and function until the late second decade. Furthermore, muscle biopsies from two affected patients show an inflammatory pattern that is mainly perivascular but also intrfascicular. Linkage analysis revealed a locus at chromosome 2p16 (D2S292, Z = 3.35, q = 0). A novel mutation producing a G to A bp alteration was identified. This mutation is predicted to affect position 5 in the intron following aa 1686, (5711 bp) of the dysferlin cDNA sequence. We found this mutation alters an acceptor site resulting in cryptic splicing and no normal dysferlin mRNA synthesis. Dysferlin is a novel protein of unknown function. Sequence comparisons suggest that dysferlin may be important for membrane fusion in muscle. Thus, splice site mutations that disrupt dysferlin appear to cause a phenotype that may be consistent with a membrane fusion defect. The etiology of the inflammatory response remains unexplained.

Program Nr: 2710 from the 1999 ASHG Annual Meeting

Towards new mouse models for Duchenne Muscular Dystrophy. *R.G.H.J. Maatman[1], E.J. de Meijer[1], J.J. Heus[2], G.J.B. van Ommen[1], J.T. den Dunnen[1]*. 1) Dept Human and Clinical Genetics, Leiden Univ Medical Ctr, Leiden, Netherlands; 2) Pharming Holding N.V., Leiden, Netherlands.

Duchenne Muscular Dystrophy (DMD) is caused by mutations in the 2.4 Mb dystrophin gene, affecting 1:3500 new born males worldwide. For DMD the mdx-mouse is the preferred model, but, for specific purposes the value of the mdx-mouse model is limited. We are currently generating a mouse model, using the Cre/loxP technology, carrying a deletion of the entire dystrophin gene and a "humanized" dystrophin mouse. Introduction of a loxP site in dystrophin's first and last exon by homologous recombination in mouse embryonic stem (ES) cells and subsequent Cre-recombinase introduction results in lines, lacking the entire dystrophin gene. The mdx0 mouse facilitates transgenic experiments directed at the elucidation of the function of individual dystrophin isoforms. In addition, it will allow to study gene therapy by providing a tool to evaluate the therapeutic potential of gene transfer protocols, especially regarding the immunogenic rejection of the expressed dystrophin. To generate a "humanized" dystrophin mouse a 2.6 Mb YAC containing the human dystrophin gene was constructed and transferred to ES cells, using spheroblast fusion. Two ES cell lines showed stable integration of the YAC without any detectable dystrophin gene rearrangements. Dystrophin expression was detected, using specific antibodies after MyoD induced in vitro differentiation. Both ES cell lines have been used to generate chimeric mice which are currently being crossed to obtain germline transmission. Analysis of one of the chimera by RT-PCR and Western blotting showed expression of the full-length human dystrophin gene in muscle tissue, proving the presence of all elements to direct tissue specific expression. Crossing these transgenic mice onto mdx0 mice lacking dystrophin will give a "humanized" dystrophin mouse, facilitating specialized studies e.g. testing protocols for gene therapy by modification of splicing in vivo.

Program Nr: 2711 from the 1999 ASHG Annual Meeting

The common CFTR deletion of Slavic descent, CFTRdele2,3(21kb), is associated with severe cystic fibrosis phenotype: a matched case control study in 30 Czech patients. *M. Macek Jr[1], T. Dörk[3], D. Zemkova[2], A. Krebsova[1], M. Koudova[1], I. Sakmaryova[1], M. Macek Sr.[1], V. Vavrova[2].* 1) Inst. Biol & Med. Genetics, 2nd Sch. Med & UH Motol, Prague, Czech Rep; 2) II. Dept. Pediatrics, Charles Univ., UH Motol-Prague, Czech Rep; 3) Inst. Hum. Genetics, MHH, Hannover, Germany.

The recently identified cystic fibrosis transmembrane conductance regulator (CFTR) gene deletion, CFTRdele2,3(21kb), spanning introns 1 to 3 (www.genet.sickkids. on.ca/cftr/) is one of the most common CFTR mutations in European populations with the current or historic Slavic presence (Dork et al. 1999)-Russia, Ukraine, Poland, Austria, Germany, Slovenia, Slovakia, Lithuania, Latvia and Macedonia in an decreasing order; range 0.9%-6.4% of all CF chromosomes. It was found in 40/628 (6.4%) of Czech CF chromosomes by using a rapid PCR-based assay. It is our 2nd most common CF allele that raised the total mutation detection rate to > 95%. In all cases it is associated with uncommon CF-linked "A" (Km19-XV2c) and STR intragenic "16-33-13" (IVS8CA-IVS17bTA-IVS17bCA) haplotypes. One patient was homozygote, 30 were compound heterozygote (CH) with DF508, while 8 cases were CH with other CF alleles (G551D, G542X, N1303K, 2143delT, R1162X, 1898+1G->A). In 30 CFTRdele2,3(21kb)/DF508 CH a matched case-control study (for sex, closest age and level of therapy) with DF508 homozygotes (DF508/DF508) was performed. Semi-longitudinal study was carried out versus a group of 151 DF508/DF508. This deletion is a severe CF allele, with all patients being PI, comparable to the DF508/DF508. However, meconium ileus (MI) and DIOS are more frequent (11/30; p=0.002), despite the comparable frequency of other GI complications. This implies that our patients were diagnosed earlier than DF508/DF508 (at 0.8+1.9 years; p=0.014), but do not have a less favorable survival than DF508/DF508. Our data indicate that patients with yet unidentified CF alleles, in particular suffering from MI or DIOS, should be screened for the CFTRdel21kb mutation. Screening for this deletion may also significantly improve prenatal and postnatal diagnosis of CF in families of Slavic origin in Canada and United States. Supported by IGA MZCR (2056-5, 2899-5, 3526-3, 4124-3); OK192.ö.

Program Nr: 2712 from the 1999 ASHG Annual Meeting

Mutational Analysis of the SMN-associated genes SmN and SIP1. *M.D. Mailman[1], K.Y. Alam[1], A.H.M. Burghes[2], T.W. Prior[1]*. 1) Department of Pathology, Ohio State Univ, Columbus, OH; 2) Department of Medical Biochemistry, Ohio State Univ, Columbus, OH.

Although we now have a highly sensitive and specific direct molecular test for 5q-SMA, the clinical diagnosis is not as straightforward. In our experience, we have found that approximately 50% of the cases sent to the molecular diagnostic lab do not have deletions of the SMN gene. These patients all share some clinical features of SMA, ranging from the more severe type I to the milder type III. Recent reports have shown that SMN interacts with SIP1 and with several of the snRNP Sm core proteins, including SmN. SIP1 colocalizes with SMN in nuclear bodies called gems and this complex is believed to be important for pre-mRNA splicing via its role in snRNA metabolism. It is possible that mutations in the proteins that interact with SMN and its biochemical pathway might produce a phenotype resembling SMA. Only the cDNA sequence of SIP1 and SmN have previously been published. Therefore, to screen patients for mutations in these genes, intron/exon borders had to be identified so that intronic primers could be used for exon screening. Intron/exon borders were identified using the vectorette PCR and long PCR techniques. Heteroduplex analysis was then used to screen individual exons for mutations. We show here that the SmN gene contains 7 exons. Thus far, we have screened 99.7% and 27% of the SmN and SIP1 coding sequences, respectively, for mutations. A rare polymorphism has been found in exon 5 of the SmN gene, however no mutations have yet been identified in 203 patients with clinical symptoms resembling SMA who are SMN-positive. Since SIP1 and SmN are among the first proteins that have been shown to directly associate with SMN, they are potential candidates for mutation analysis. However, mutations have not been identified. Therefore, we believe that mutations in these genes are not a predominant cause of an SMA-like phenotype.

Program Nr: 2713 from the 1999 ASHG Annual Meeting

A novel frameshift mutation in the ATP7B gene in Saudi Wilson disease patients. *R. Majumdar[1], M. Al Jumah[1], S. Al Rajeh[2], A. Awada[1], A. Al Zaben[1], A. Al Traif[1], M. Fraser[3], S. Al Uthaim[1], A. Snellen[1], M. Paterson[3].* 1) Division of Neurology, Dept of Medicine, King Fahad Natl Guard Hosp, Riyadh, Saudi Arabia; 2) Division of Neurology, King Saud University, Riyadh, Saudi Arabia; 3) Department of Biological and Medical Research, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia.

Wilson disease (WD) is an autosomal recessive disorder of copper metabolism culminating in liver, brain, and kidney damage. ATP7B, the gene mutated in WD, contains 21 exons and encodes a copper transporting ATPase. Numerous mutations, all of which are located in the first twenty exons have been reported among different ethnic groups. In this study, we have screened for mutations in the ATP7B gene in fifteen Saudi WD patients representing different tribes. In all patients, the entire gene coding sequence, in addition to the intron-exon boundaries, were screened for alterations by conventional mutation detection enhancement (MDE) heteroduplex analysis, followed by direct sequencing of the regions that showed heteroduplex formation. A novel homozygous mutation (4193delC) in exon 21, causing a frameshift leading to premature truncation of the protein, was detected in seven of fifteen patients. This mutation was not found in a total of 55 normal Saudi subjects (i.e. 110 chromosomes). To our knowledge, this is the first time a mutation has been detected in exon 21 of ATP7B (hydrophilic C-terminus of the open reading frame). The finding that all seven Saudi WD patients from different tribes have consistently shown the same mutation in exon 21 of the ATP7B gene, strongly suggests that 4193delC is a unique mutation for Saudis, especially that this mutation was not described in other ethnic groups. This finding may also provide insight into the function of the long stretch of the C-terminal hydrophilic domain on the ATP7B protein. (This work was supported by KACST Project AT 18-03, Saudi Arabia).

Program Nr: 2714 from the 1999 ASHG Annual Meeting

Novel *FGFR2* mutation associated with a partial Apert syndrome phenotype. *D.K. Manchester[1,2], J.K. Allen[1,2], M. Handler[3], F.V. Schaefer[4].* 1) Div Genetics Services, The Children's Hosp, Denver, CO; 2) University of Colorado Health Sciences Center, Denver, CO; 3) Rocky Mountain Pediatric Neurosurgery,P.C., Denver, CO; 4) H.A. Chapman Institute of Medical Genetics, Oklahoma City, OK.

Several distinct phenotypes with craniosynostosis - Crouzon, Apert, Pfeiffer and Jackson-Weiss syndromes - are associated with missense mutations in the human gene for Fibroblast Growth Factor Receptor 2 (FGFR2), a transmembrane tyrosine kinase receptor with three extracellular immunoglobulin(Ig)-like domains. The mechanisms responsible for these phenotypes are not understood. Missense mutations in the linker region between the second and third extracellular Ig domains of FGFR2 are associated with Apert syndrome (AS, acrocephalosyndactyly). We have ascertained a patient with a partial AS phenotype in whom we have detected a novel missense mutation outside the Ig-like loops of FGFR2. This female infant presented at birth with facial features (hypertelorism; high, broad, flat forehead; downslanting palpebral fissures; midface hypoplasia; depressed nasal bridge with beaked nose) and radiographic findings (bilateral coronal synostosis with expansion of the temporal regions; small cranial base; unilateral anomalies of the cochlea, middle ear ossicles and external canal; fusion of cervical vertebra, C5-C6) felt to be typical of AS. Hands, however, had no syndactyly. Fingers were tapering and there were unusual palmar creases but thumbs were normal. She developed hydrocephalus at 4 months. Molecular analysis of *FGFR2* performed by PCR amplification of exons 7 and 9 and DNA sequencing (ABI model 373) revealed her to be heterozygous for a C to T transition changing codon 355 from alanine to valine (ala355-to-val). Parents had normal examinations and neither carried the ala355-to-val variant. No point mutations in *FGFR1* and *3* were found in any of the three family members. Anderson *et al.* (1998, *Hum. Molec. Genet.* 7: 1475-1483) have presented evidence that missense mutations previously associated with AS affect FGFR2 dissociation kinetics *in vitro*. The apparent novel mutation found in this patient would also be expected to have limited impact on overall protein structure.

Program Nr: 2715 from the 1999 ASHG Annual Meeting

Emerin is associated with lamin A/C in one subset of kidney cell nuclei while lamin B1 is present in the emerin-negative nuclei. *S. Manilal, N.T. Man*, C.A. Sewry, G.E. Morris.* MRIC Biochemistry Group, N. E. Wales Institute, WREXHAM, UK.

The X-linked form of Emery-Dreifuss muscular dystrophy (EDMD) is caused by mutations in the nuclear membrane protein emerin, while mutations in lamin A/C of the nuclear lamina are responsible for most cases of autosomal dominant EDMD. The two forms have a similar phenotype, with cardiac and skeletal muscle affected, suggesting a functional relationship between the two proteins. Direct interaction between emerin and lamin A/C has not yet been demonstrated, but remains a strong possibility.

In an earlier study (Manilal et al, Hum. Mol. Genet. 8 (1999) 353), lamin B1 was virtually undetectable immunohistochemically in emerin-positive cardiomyocyte and skeletal muscle nuclei, but levels of this protein were high in nuclei without emerin. We now present evidence supporting an association of emerin with lamin A/C, but not lamin B1, in the pig kidney. Triple-labelling was performed using a rabbit antiserum against emerin, mouse monoclonal antibodies against lamins and a DAPI stain for nuclei. In kidney glomeruli, about half the nuclei stained for both emerin and lamin A/C, but not lamin B1, and the other half stained for lamin B1 only. Kidney tubule nuclei were emerin-negative and lamin B1-positive. Similar mutual exclusivity was observed in other tissues, such as spleen, heart and skeletal muscle, but not in all tissues. It is not yet clear whether absence of immunostaining is due to absence of the antigen or epitope masking by other nuclear proteins. However, EDMD carrier skin biopsies, in which 50% of cells are emerin null mutants, showed no variation in lamin immunostaining, which shows that emerin does not mask the lamin B1 epitope.

The results are consistent with functional interaction between emerin and A-type lamins. The absence of lamin B1 staining is not restricted to the affected tissues in EDMD.

We thank the British Heart Foundation for grant support (PG97142) and Yves Raymond (Montreal) for lamin mAbs..

Program Nr: 2716 from the 1999 ASHG Annual Meeting

Extrachromosomal Amplification of a *CFTR* YAC in a Cystic Fibrosis Airway Epithelial Cell Line. *M.K. Mansoura, K.A. Henning, E.A. Novotny, S.T. Compton, A.P. Chetty, M.A. Ashlock.* Genetics and Molecular Biology Branch, NIH/NHGRI, Bethesda, MD.

We attempted to correct the cystic fibrosis (CF) phenotype in CF airway epithelial cell lines by introducing the full-length 250 kb *CFTR* gene and 300 kb of 5' flanking DNA in a YAC containing a neo^R gene for selection in mammalian cells. Two restriction sites have been engineered into the 3' untranslated region (UTR) of the *CFTR* gene, allowing for the unique identification of YAC-derived *CFTR* DNA and RNA. Stable neo^R CFT1 (n=5)and IB3-1 (n=7) CF airway epithelial clones were derived by transfection of gel-purified YAC DNA. Southern blot analysis indicated that the 3' UTR of *CFTR* derived from the YAC was present at single copy in the IB3 clones. However, this region of the gene was amplified greater than 10-fold in the three CFT clones analyzed thusfar. This level of amplification was present after more than 20 passages in culture but diminished significantly, but not completely, when the cells were taken off selection. Fluorescent in-situ hybridization (FISH) analysis revealed a single site of integration in each of the IB3 clones. In striking constrast, the *CFTR* YAC was present in extrachromosomal elements in the 3 CFT clones. In addition to the somewhat heterogeneous size appearance of these signals, the number of signals varied from metaphase-to-metaphase (n=0 to >50) in these clones indicating that they were not mitotically stable. Pulsed-field gel electrophoresis of one of these CFT clones in the presence and absence of gamma irradiation indicated that the extrachromosomal elements were most likely circular and greater than 6 MB in length. Future experiments are designed to further identify (1) the content and stability of these extrachomosomal elements, and (2) the critical factors which led to their generation. Specifically, to address the latter, we have repeated the transfection experiment in the same two cell lines using a comparably-sized YAC which does not contain the *CFTR* gene. Neo^R clones from each cell line have been derived and are being expanded in culture for future analysis.

Program Nr: 2717 from the 1999 ASHG Annual Meeting

Genetic and transcritpion analysis in Rett syndrome. *E. Manzati[1,2], S. Bigoni[1], F. Gualandi[1], C. Scapoli[3], M. Guarna[4], G. Pini[5], M. Zappella[4], F. Muntoni[2], G. Hajek[4], E. Calzolari[1], A. Ferlini[1,2].* 1) Istituto di Genetica Medica, Universita' di Ferrara, Ferrara, Italy; 2) Neuromuscular Unit, Imperial College School of Medicine, Hammersmith Campus, London, UK; 3) Sezione di Biologia Evolutiva, Universita' di Ferrara, Ferrara, Italy; 4) Neuropsichiatria Infantile, Policlinico Le Scotte and Istituto di Istologia, Universita' di Siena, Siena, Italy; 5) Servizio di Neuropsichiatria Infantile, Azienda USL12, Viareggio, Italy.

Rett syndrome (RS), a severe progressive neurodegenerative disease, is predominantly sporadic but the existence of several families with recurrence of the disease reinforces the view that this pathology represents a genetic entity. The evidence that RS affects females only has also suggested an X-linked dominant inheritance and linkage analysis has recently mapped the RS locus to Xq28. In order to investigate the molecular defect of this disease we performed transcription analysis on an autoptic specimen from a RS brain cortex as well as on several normal adult and foetal cerebellum and brain tissues. We have studied the transcription pattern of the following four known genes, previously mapped to Xq28: interleukin 9 receptor gene (IL9R), host cell factor 1 gene (HCF1), melatonin-related receptor gene (GPR50), putative new subunit class (e) of the GABA-A neurotransmitter receptor gene (GABRE). Total RNA was extracted from frozen tissues, RT-PCR method was carried out using four pairs of oligonucleotides designed to amplify specific regions of the candidate genes transcripts. The RS brain cortex exhibited an identical transcription pattern to that found in the normal tissues. In order to confirm the Xq28 mapping, we collected DNA from a very large Italian pedigree with RS recurrence, already reported by our group. A preliminary segregation analysis showed as both the autosomal recessive and the X-linked model of inheritance are compatible with the pedigree configuration. We are studying this pedigree using several polymorphic markers mapped in Xq28. In addition we are exploring in this family a polymorphic (CAG)n repeat located in exon 9 of IL9R gene both for linkage analysis and for evaluating the possibility of a triplet repeat expansion mutation.

Program Nr: 2718 from the 1999 ASHG Annual Meeting

Mutation analysis of Krabbe Disease and Metachromatic Leukodystrophy in Portugal. *A.M.L. Marcao, O.M.O. Amaral, E.M. Pinto, M.C. Sa Miranda.* Genetic Neurobiology, IBMC- Univ.of Porto, Porto, Portugal.

The molecular characterisation of Portuguese MLD and KD patients was carried out in order to identify the molecular lesions underlying the biochemical deficits. In the case of MLD, the application of PCR-RFLP and PCR-SSCP analysis allowed a 100% rate of mutation identification; eight different mutations were identified. The I allele was found at a very high frequency (64%), whereas the A allele represented only 3,5% of the mutated MLD alleles. As expected, all the homozygotes for the I allele presented the late infantile form, making it much more frequent than the juvenile and adult forms. Two previously described missense mutations (I179S; D255H), here associated to adult and late infantile MLD were both found in heterozygosity with allele I. Two new missense mutations (C300F; P425T) were found to be associated to late infantile and juvenile forms and two novel microlesions (g.2408delC and g.1190-1191insC) were identified in two late infantile patients. The haplotypes previously proposed for I, A, I179S and D255H mutations were confirmed, suggesting the possible existence of common ancestors. With regard to KD, although all patients presented the infantile form, large molecular heterogeneity was found. In this case, the approach followed consisted on RT-PCR-SSCP analysis. In addition to the five mutations previously identified, in a collaborative study with Dr. Wenger, four other mutations and several polymorphic changes were found. Of the nine mutations identified four led to deletions, one was nonsense and four were missense mutations, three alleles remain undefined. It should be noted that only one of the deletions (del30-kb, ex11-17) and one of the missense mutations (T513M) are known to be common in North European KD patients, all other mutations seem to be private and appear in various polymorphic backgrounds. As described for patients with North European ancestry, mutations del30Kb and T513M appeared associated with polymorphisms 502T and 694A, respectively. The molecular findings here reported may provide useful information for the study of other populations, since different mutation profiles were found in Portuguese patients.

Program Nr: 2719 from the 1999 ASHG Annual Meeting

Mutation Screening in the CYP1B1 Gene of German Primary Congenital Glaucoma Patients. *C.Y. Mardin[1], M. Zenker[2], U. Mayer[1], G.O.H. Naumann[1], B. Rautenstrauss[2], K. Michels-Rautenstrauss[2].* 1) Department of Ophthalmology, FAU Erlangen-Nuremberg, Erlangen, bavaria, germany; 2) Institute of Human Genetics, Erlangen, Germany.

Autosomal recessive primary congenital open-angle glaucoma (buphthalmus) is caused by developmental defects of the trabecular meshwork and the anterior chamber of the eye. Two chromosomal locations on 2p21 (GLC3A) and 1p36 (GLC3B) are known to be associated with this condition. For GLC3A-linked families a series of mutations in the cytochrome P4501B1 (CYP1B1) gene were found to be the predominant cause of buphthalmus. One half of the known mutations, including 1 nonsense, 6 frameshifts and one large deletion are predicted to truncate the CYP1B1 open reading frame, whereas the other half are missense mutations in highly conserved positions. A mutation screening in buphthalmus patients by direct sequencing of exon 2 and 3 of the CYP1B1 gene revealed a novel mutation in exon 3. A newborn male with opaque corneae was detected to show on the forth day after birth intraocular pressures under general anaesthesia of 34/37 mmHg (R/L), Haab's lines, axial lengths of 20.6 mm and corneal diameters of 13.0x12.0 mm. No family history for glaucoma was known. The parents were consanguinous. The homozygous missense mutation Arg355Stop found, truncates the protein by 188 aminoacids including the functionally essential hem binding site. Furthermore we identified five of the six known polymorphisms. Thus our results confirm the crucial role of CYP1B1 in primary congenital glaucoma.

Program Nr: 2720 from the 1999 ASHG Annual Meeting

A null a**1(V) collagen allele in a family with type II Ehlers-Danlos Syndrome causes variation in type I collagen fibril diameter.** *J.C. Marini[1], P. Bouma[1], W.G. Cole[2], J.B. Sidbury[1]*. 1) Sect Connective Tissues Disord, HDB/NICHD/NIH, Bethesda, MD; 2) Division of Orthopaedics, The Hospital for Sick Children, Toronto, Ontario M5G1X8, Canada.

Splicing defects in the a1 and a2 chains of type V collagen have been identified in several families with EDS I-II. We studied a three generation family with EDS II. They show skin laxity, small joint hypermobility, easy bruising and occasional cigarette paper scars. RT-PCR amplification using primers in exons 9 and 28 of a1(V) cDNA yielded normal and larger products from the proband. Sequencing revealed a 100 bp insertion from the 3'end of intron 13 between exons 13 and 14. The genomic defect was identified as an A®G substitution at the -2 position of the acceptor site of exon 14. A cryptic acceptor site -100 bp within intron 13 is used as the new acceptor splice site. The insertion shifts the reading frame +1 and results in a stop codon within exon 17. To determine the level of mutant a1(V) mRNA, we did RNA protection assays and Northern slot blot with ASO. RPA of proband total RNA showed that the mutant a1(V) mRNA was 7-9% of the level of normal a1(V) mRNA. ASO blots yielded proband levels of normal a1(V) mRNA that were about half of the control. This suggests that the mutation causes a null allele with nonsense mediated decay. Proband fibroblasts secreted 65% the amount of a1(V) chain secreted by controls. Type V collagen incorporates into type I collagen fibrils in the ECM and regulates fibril diameter. Scanning electron micrographs of type I collagen fibrils in proband dermal biopsy showed variation in fibril diameter. Two to five percent of fibrils have a smaller diameter and occasional fibrils have a cauliflower configuration. These abnormalities are the same type seen in EDS patients with exon skipping defects, but at lower frequency. This case demonstrates that a reduction in the amount of normal a1(V) collagen is sufficient to cause symptoms of mild EDS II. Similar to the relationship seen for type I collagen defects and osteogenesis imperfecta, the null allele in this family appears to cause milder clinical features than seen in cases with structural alterations in type V collagen.

Program Nr: 2721 from the 1999 ASHG Annual Meeting

Molecular Characterization of Congenital Glaucoma in an Inbred Community: a step towards preventing glaucoma-related blindness. *S. Martin[1], J.E. Sutherland[3], A.V. Levin[2,3], E. Héon[1,2,3,4].* 1) Eye Research Institute of Canada; 2) University of Toronto, Department of Ophthalmology; 3) The Hospital for Sick Children, Department of Ophthalmology; 4) The Hospital for Sick Children Research Institute, Toronto, Canada.

Glaucoma constitutes a leading cause of irreversible blindness in Canada. Congenital glaucoma usually manifests during the first five years of life and is characterized by severe visual loss and autosomal recessive inheritance. Four congenital glaucoma loci have been identified on chromosome 1p36, 2p21, 6p25 and 4q25 respectively. A branch of a large inbred Amish community from Southwestern Ontario was affected with congenital glaucoma and studied to identify the glaucoma-related genetic defect. Members of a 4 generation family were examined and the attribution of the genotypes was done masked from the disease status. Linkage analysis was performed using the MLINK component of the LINKAGE package (v.5.1). Mutational analysis of the candidate gene was done by direct cycle sequencing, restriction enzyme digestion and fragment analysis. Evidence for linkage was found with markers in the 2p21 region with a maximum LOD score 3.34 with markers D2S1348 and D2S1346. Mutational analysis of the candidate gene CYP1B1 identified two different disease causing mutations in exon 3 not seen in a control population of 100 individuals. The congenital glaucoma phenotype for this large inbred Amish family appears to be due to mutations in CYP1B1 (2p21). The identification of these molecular defects will be used to identify carriers of the CYP1B1 mutation and those who are at high risk of developing glaucoma in that community. Other Amish communities should be assessed for these mutations. This will improve genetic counseling and early identification of glaucoma and hopefully minimize glaucoma-related visual loss

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Program Nr: 2722 from the 1999 ASHG Annual Meeting

Mutation detection by TaqMan-allele specific amplification: application to molecular diagnosis of glycogen storage disease type Ia and medium-chain acyl-CoA dehydrogenase deficiency. *Y. Matsubara[1], K. Fujii[1,2], J. Akanuma[1], K. Takahashi[1], S. Kure[1], Y. Suzuki[1], P. Rinaldo[3], K. Narisawa[1]*. 1) Department of Medical Genetics; 2) Department of Pediatrics, Tohoku University School of Medicine, Sendai, Japan; 3) Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester MN.

We have devised an allele-specific amplification method with a TaqMan fluorogenic probe (TaqMan-ASA) for the detection of point mutations. A target sequence containing a mutation site was PCR-amplified with two sets of allele-specific primers, one complementary to the wild-type sequence and the other complementary to the mutant sequence, in the presence of a TaqMan fluorogenic probe. The TaqMan probe, which hybridized to the template in between the two PCR primers, was labeled with a reporter dye at the 5'-end and a quenching dye at the 3'-end. During PCR with a matched primer set, Taq DNA polymerase synthesizes a new strand and cleaves the reporter dye, thus increasing the intensity of the fluorescence signal. The level of fluorescence was monitored on a fluorometer in real-time during PCR to determine the "threshold cycle" at which the fluorescence exceeds a baseline. A difference in the amplification efficiency between two separate PCR reactions was determined from the difference of "threshold" cycles, thus differentiating mutant and normal alleles. Since the method measured the efficiency of amplification rather than the presence or absence of end-point PCR products, false amplification of mismatched template did not interfere with the interpretation. Therefore it allowed greater flexibility in designing allele-specific primers and an ample technical margin for allelic discrimination. We applied the TaqMan-ASA method to detect a prevalent g727t mutation in Japanese patients with glycogen storage disease type Ia and a common a985g mutation in Caucasian patients with medium-chain acyl-CoA dehydrogenase deficiency. The method requires no post-PCR processing, can be automated and may be applicable to the DNA diagnosis of various genetic diseases.

Program Nr: 2723 from the 1999 ASHG Annual Meeting

Germline mosaicism of a partial *DCX* deletion causes lissencephaly in a son and subcortical band heterotopia in a daughter. *N. Matsumoto[1], S. Mewborn[1], J.A. Kuc[1], C. Schanen[2], B.F. Crandall[3], W.B. Dobyns[1], D.H. Ledbetter[1]*. 1) Department of Human Genetics, The University of Chicago, Chicago, IL; 2) Departments of Human Genetics and Pediatrics, UCLA School of Medicine, Los Angeles, CA; 3) Departments of Psychiatry and Biobehavioral Sciences and Pediatrics, UCLA School of Medicine, Los Angeles, CA.

Classical lissencephaly (LIS) and subcortical band heterotopia (SBH) are related malformations of neuronal migration. *LIS1* (*PAFAH1B1*) at 17p13.3 and *DCX* (*XLIS*) at Xq22.3-q23 are the two LIS and SBH-causative genes isolated to date, and cause different patterns of malformations (Pilz et al.; Hum Mol Genet;1998;7:2029-2037). *DCX* mutations have been found in sporadic LIS males, sporadic SBH females, and families with both SBH females and LIS males.

We evaluated a family with a LIS son and SBH daughter. The son had hypotonia and feeding problems at birth, seizures at 2 months, and a gastrostomy tube at 2 years. Cranial CT scan at age 1 week showed diffuse LIS and enlarged lateral ventricles. At 7 years, he had profound mental retardation and occasional seizures. The girl presented with developmental delay and seizures in the first year of life. Examination at 4 years showed normal head size, borderline hypertelorism, and mild mental retardation. Cranial MRI at 4 years showed bilateral SBH of medium width. The mother of the two children has normal intelligence and no history of seizures. No brain imaging was performed.

PCR analysis indicates that *DCX* exons 6 through 9 are deleted in the boy with LIS. Southern analysis demonstrates that three restriction fragments are deleted in the LIS male and that the same fragments show a 50% reduction in intensity in the SBH female. These three restriction fragments are present at the control level of intensity in the mother. These results are strongly suggestive of maternal germline mosaicism, although we cannot completely exclude the possibility of low level somatic mosaicism. We also studied X chromosome inactivation patterns in this family, and found no significant skewing in the peripheral blood of the SBH female.

Program Nr: 2724 from the 1999 ASHG Annual Meeting

Screening of autosomal dominant recurrent fever families for TNFR1 mutations. *M. McDermott[1], I. Aksentijevich[2], E. Aganna[1], L. Karenko[3], E.M. McDermott[4], A.I. Hoepelman[5], M. Phelan[1], A-M. Teppo[3], T. Pettersson[3], K.A. Quane[1], R.J. Powell[4], M.G. Molloy[1], B.W. Ogunkolade[1], E.J. Zweers[5], D.L. Kastner[2], G.A. Hitman[1].*
1) Medical Unit, Royal London Hosp, London, England; 2) ARB/NIAMS, NIH, Bethesda, MD, USA; 3) Depts. of Dermatology (LK, AR), and Int. Medicine (TP, AT), Helsinki University Hospital, Helsinki, Finland; 4) Clinical Immunology Unit, Queen's Medical Centre, Nottingham, UK; 5) Bronovo Ziekenhuis, The Hague, Holland.

We have recently described a range of missense coding mutations of tumor necrosis factor receptor 1 (TNFR1) in autosomal dominant TNFR-1 associated periodic syndromes or TRAPS (Cell 97:133). The majority of these mutations disrupt disulphide bonds in the extracellular domains. We have screened a further 3 families (Dutch, Finnish and Irish) with autosomal dominant recurrent fevers (ADRF) as well as 2 sporadic cases for TNFR1 mutations. Sequencing of TNFR1 in the proband daughter and father of the Dutch family revealed a G to C transversion producing an arginine to proline mutation at residue 92 (R92P). All members except the mother in this family were found to have half the normal levels of soluble TNFR1 in their sera. An RFLP assay confirmed a mutation in all family members except the mother. There are 2 unaffected carriers in the family and this non-cysteine mutation appears to be less penetrant than those involving cysteine residues. In the Northern Finns and Irish families we have sequenced the TNFR1 coding region but did not identify any mutations. These families are therefore either TRAPS negative or have mutations in the TNFR1 regulatory regions, which we are currently investigating. The Finnish family has features typical of TRAPS but, based on history, a diagnosis of familial cold urticaria is more likely in the Irish family. Finally the 2 sporadic cases whose symptoms responded to steroids were also mutation negative. These results indicate some degree of genetic heterogeneity in ADRF and it is possible that in isolated individuals where the disease phenotype is not transmitted a TRAPS-like condition may be polygenic or have arisen through non-genetic causes.

Program Nr: 2725 from the 1999 ASHG Annual Meeting

Novel and disease-related mutations in the HEXA gene in non-Jewish individuals. *M.J. McGinniss[1, 2], D.H. Brown[2], A. Fulwiler[1], M. Marten[1], M.M. Kaback[2].* 1) Molecular Genetics Lab, #5031, Children's Hosp, San Diego, CA; 2) Department of Pediatrics University of California, San Diego.

Tay-Sachs disease (TSD) is a neurodegenerative disease that is inherited in an autosomal recessive fashion. Mutations that abolish the activity of the Hex A enzyme encoded by the HEXA gene are associated with the more severe and usually lethal infantile form of TSD. Individuals in the California TSD carrier screening program that do not carry one of the four common disease-associated mutations or one of the two common pseudo-deficient alleles, but that are enzymatically defined as carriers, have been screened for the presence of novel mutations. To date more than 70 mutations have been associated with infantile TSD and we report here 5 novel mutations that appear to be disease-related. DNA samples from enzymatically defined carriers that were negative for both the disease-associated and the two pseudo-deficient mutations were analyzed by SSCP. DNA was amplified from all coding regions of the HEXA gene and electrophoretic variants evident from SSCP were sequenced revealing 5 novel and potentially deleterious mutations. All of these mutations were found in non-Jewish individuals of European or Hispanic ethnic origin and included: two single base pair deletions (1258delT, 1499delT), a nonsense mutation (Ser52Stop), and two missense mutations (His318Arg, Pro439Ser). All 5 mutations are almost certainly disease-related since three are evident in obligate carriers, one involves a frameshift with a stop codon downstream, and the remaining one involves a nonconservative amino acid change in an enzymatically-defined carrier. Identification of these novel mutations adds to the extensive mutational spectrum for the HEXA gene, and contributes to more accurate carrier identification, diagnosis and prognosis for TSD. In addition, these mutations are candidates for further studies of gene expression and to elucidate the structure function relationships of the heterodimeric Hex A (ab) isozyme. (Supported in part by a contract from the Genetic Disease Branch, Department of Health, State of California.).

Program Nr: 2726 from the 1999 ASHG Annual Meeting

Deletion of a branch-point consensus sequence in the LMX1B gene causes exon skipping in a family with nail patella syndrome. *I. McIntosh, J.D. Hamlington, M.V. Clough, J.A. Dunston.* Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD.

Nail patella syndrome (NPS) is a pleiotropic condition characterized by dysplasia of the nails, hypoplasia of the patellae, elbow dysplasia and progressive kidney disease. The syndrome is inherited in an autosomal dominant manner and has been shown to result from mutations in the LIM-homeodomain encoding LMX1B gene. The LMX1B transcription factor plays a role in defining the development of dorsal specific structures during limb development; its role in other organs is unclear. Analysis of over sixty LMX1B mutations in NPS families supports the hypothesis that the syndrome results from haploinsufficiency due to loss of function mutations. During the search for LMX1B mutations causing NPS, a 17bp deletion was identified upstream of the 3' splice consensus which removed a canonical branchpoint sequence within the first intron. This deletion was found to segregate with the NPS phenotype in 14 individuals in a single, large family, and was absent from 11 unaffected relatives. Analysis of RNA extracted from skin fibroblasts from an individual carrying the mutation revealed two distinct LMX1B transcripts: one of normal sequence, the other lacking exon 2. Mutations of branchpoint sequences have been shown previously to result in cryptic splicing and intron retention, as well as exon skipping. In this instance, it is hypothesized that the absence of a branchpoint sequence prevents stable binding of the U2 snRNP during spliceosome assembly presumably destabilizing the interactions of U2AF and U1 with the 3' and 5' splice sites. Since these factors are required for definition of the downstream exon (exon 2), the splicing machinery would join exon 1 to exon 3. Skipping of exon 2 disrupts the reading frame leading to premature termination within exon 3. Any polypeptide synthesized from this transcript would lack all functional domains and would be predicted to be non-functional. This is in agreement with the haploinsufficiency mechanism suggested previously.

Program Nr: 2727 from the 1999 ASHG Annual Meeting

Hypotrichosis of Marie Unna maps between D8S258 and D8S298: Exclusion of the hairless gene by FISH on combed DNA. *W.H.I. McLean[1], R. Ekong[2], C.M. Coleman[1], F.J.D. Smith[1], M.A.M. van Steensel[3], I. Kluijt[3], H.P. Stevens[3], A. Messenger[3], J. Uitto[4], C. Schurra[5], A. Bensimon[5], M.S. Povey[2], P.M. Steijlen[3].* 1) Epithelial Genetics Group, Human Genetics Unit, University of Dundee, Dundee, Scotland, UK; 2) MRC Human Biochemical Genetics Unit, London; 3) Depts Dermatology and Clinical Genetics, Nijmegen, Amsterdam, Sheffield and London; 4) Dept Dermatology, Jefferson Medical College, Philadephia; 5) Institut Pasteur, Paris.

Hypotrichosis of Marie Unna (MU) is an autosomal dominant form of alopecia without other ectodermal abnormality. Following genomewide linkage analysis using large Dutch and British kindreds, a maximum combined 2-point lod score of 13.42 at q = 0, was obtained using marker D8S560 on chromosome 8p. Informative recombinants placed the gene between D2S258 and D8S298. The human homologue of the murine hairless gene (*hr*), in which recessive loss-of-function mutations have been shown to cause congenital atrichia, had previously been mapped to this vicinity. Radiation hybrid mapping with the Stanford G3 panel placed this candidate gene 7 $cR_{10,000}$ from D8S298 but was unable to resolve the order of these markers. No mutations were identified in *hr* using strategies based on either cDNA or genomic DNA. However, there remained the possibility of there being unknown alternate exons or transcription initiation sites for the gene. BAC clones were obtained from the Research Genetics library, corresponding to D8S560, *hr* and D8S298. FISH analysis on combed total human genomic DNA using these BAC clones placed the *hr* clone at an estimated distance of 124-146 kb outside of the interval between D8S560 and D8S298, formally excluding the involvement of this gene in MU. The distance between the BAC clones corresponding to D8S560 and *hr* was determined to be 264-285 kb, with an overall mapped distance of 440-460 kb, representing an increase in the distance over which markers have been ordered on combed DNA. Currently, we are constructing a physical map of this locus to enable positional cloning of the MU gene, whose function may shed light on the molecular pathogenesis of alopecia in humans.

Program Nr: 2728 from the 1999 ASHG Annual Meeting

Mutation analysis of the *NF1* gene in 62 patients using an optimized protein truncation test (PTT), heteroduplex analysis (HA) and karyotyping allows to identify 93% of mutations. *L.M. Messiaen[1], T. Callens[1], G. Mortier[1], F. Speleman[1], D. Carton[2], M. Craen[2], J. Leroy[2], M. Abramowicz[3], F. Hayez[3], N. Van Regemorter[3], M. Bonduelle[4], A. De Paepe[1]*. 1) Dept Med Genetics, Univ Hosp Gent, Belgium; 2) Dept Pediatrics, Univ Hosp Gent, Belgium; 3) Service de Genetique, Hopital Erasme Brussels, Belgium; 4) Center Med Genetics, Vrije Universiteit Brussels, Belgium.

NF1 is characterised by neurofibromas, cafe-au-lait spots, freckling and Lisch nodules. Additional complications are often seen. Mutation analysis in the *NF1* gene has been difficult. Mutation detection rates largely depend on the efficiency of the techniques used as well as on the correct clinical diagnosis of the patients included.

We performed mutation analysis in 62 unrelated NF1 patients: 28 familial and 34 sporadic cases. Only patients fulfilling NIH diagnostic criteria were admitted. Clinical data were recorded using the NNFF International NF1 Genetic Analysis Consortium Submission Form. For all patients, we established an EBV lymphoblastoid cell line and extracted gDNA and total RNA. We analysed the complete coding region by an optimized PTT. Truncated fragments were characterised by cDNA and gDNA sequencing. If no truncated fragment was present, HA of all exons and a karyotype was performed. Thus we characterised 58 mutations (93%), including 35 novel ones. 51 mutations were found by PTT (82%), 6 by HA (10%) and 1 translocation t(14;17)(q32;q11.2) by karyotyping. 15 mutations occurred at CpG dinucleotides. 18 mutations resulted in splicing errors, only 4 of them involving the AG splice acceptor or GT donor nucleotides. Other splicing errors were caused by specific nonsense mutations, creation of novel intronic/exonic splice donor or acceptor sites, or mutations in less conserved regions of splice sites. By HA we identified 3 novel missense mutations and 3 recurrent in frame deletions of 3-6 nucleotides. All 4 patients in which the mutation remains unknown are sporadic and somatic mosaicism might underly their disorder. Deletion analysis by FISH and Southern blotting is in progress.

Program Nr: 2729 from the 1999 ASHG Annual Meeting

Mutations in chondrodysplasia punctata, X-linked dominant type (CDPX2). *A.B. Metzenberg[1], R. Kelley[2], D. Smith[1], K. Kopacz[3], R. Sutphen[4], L. Sheffield[5], G. Herman[3]*. 1) Biology, Cal State Northridge; 2) Kennedy Krieger Institute, Johns Hopkins University; 3) Children's Hospital, The Ohio State University; 4) All Children's Hospital, St. Petersburg, FL; 5) The Murdoch Institute, Australia.

Chondrodysplasia punctata comprises a group of heterogeneous disorders affecting skin and bone development. Abnormal cholesterol synthesis has recently been found in patients with the X-linked dominant form, CDPX2, and mutations in the implicated EBP gene (a putative isomerase gene) on Xp11.22-23 have been found in some individuals with CDPX2. Here we present a range of mutations in the EBP gene in 15 unrelated individuals with CDPX2, and note the prevalence of CDPX2-associated EBP mutations in Exon 2, and the existence of two potential mutational hotspots. Direct sequencing of the EBP gene from 15 of 16 unrelated individuals with a clinical diagnosis of CDPX2 revealed mutations in several exons. There were 12 patients with mutations in exon 2, including 5 nonsense mutations, 3 frameshifts resulting in early termination, 3 missense mutations, and one in-frame deletion. One missense mutation was found in Exon 3, one nonsense mutation in Exon 5, and none in one patient. One of the patients with a frameshift mutation in Exon 2 was a male, raising the possibility of a postzygotic mutation. We observed identical C->T transition mutations in a CG dinucleotide in Exon 2 in three individuals, supporting the notion of a mutational hotspot via deamination of a methyl C. Another CG->TG mutation was observed in Exon 2, which may identify a second mutational hotspot. There is no indication of a clear genotype-phenotype correlation for either the biochemical or physical abnormalities. Mutations in regulatory regions of the EBP gene or in other cholesterol biosynthetic genes have not been excluded in patients for whom no mutation was found. The CDPX2 phenotype resembles that of the bare patches mouse, which has mutations in the Xq28-syntenic Bpa gene that encodes a 3b-hydroxysteroid dehydrogenase, found in post-squalene cholesterol biosynthesis. However, no mutations have been found in the human Bpa gene thus far. bb.

Program Nr: 2730 from the 1999 ASHG Annual Meeting

Phenotype analysis in patients with a *JAGGED1* mutation. *M. Meunier-Rotival[1], C. Crosnier[1], C. Driancourt[1], N. Raynaud[1], O. Bernard[2], M. Hadchouel[1,2].* 1) INSERM U347, Kremlin-Bicetre, France; 2) Hopital de Bicetre, France.

The Alagille syndrome (MIM 118450, AGS) is an autosomal dominant disorder characterized by 5 main features: intrahepatic cholestasis, pulmonary artery stenosis, butterfly-like vertebrae, posterior embryotoxon and peculiar facies. Three of the 5 features allow the diagnosis of AGS. There are also minor features including other vascular or skeletal abnormalities, renal malformation or dysfunction, mental retardation and high-pitched voice. The AGS disease gene is *JAGGED1*. By SSCP and sequencing, mutations have been detected in 75 probands (65% of AGS patients tested). Only 19 (31%) among 62 of these cases are inherited, with no paternal or maternal bias. We studied the genotype/phenotype correlation within families.

The characteristics of 6 sibling pairs show the variability in the expressivity of the syndrome in terms of both number and severity of the features, whatever the mutation type. Similarly, a parent with a mild phenotype could have an affected child with a complete syndrome. The 7 families in which missense mutations were transmitted (of a total of 14 missense mutations corresponding to 19% of all mutations found in this work), were studied. The carrier parent had either no AGS feature (L79H, R184H), or subclinical features (R184G, W288C, C731S) or were patients with AGS (P129R, C284F). The presence of a mutation in *JAGGED1* allowed the discrimination between inherited cases of AGS, sporadic cases and cases which were erroneously presumed to be transmitted, due to familial data.

A missense mutation (C753R) in *JAGGED1* was found in an individual with an isolated paucity of intrahepatic bile ducts. Mutations in *JAGGED1* have previously been shown in patients presenting with tetralogy of Fallot. Therefore isolated hepatic defects could also be due to mutations in *JAGGED1*.

Program Nr: 2731 from the 1999 ASHG Annual Meeting

GLC1A-Locus: Mutation Screening in MYOC/TIGR and COX-2 of German POAG Patients. *K. Michels-Rautenstrauss[1], C.Y. Mardin[2], D. Schweitzer[1], S. Oezbey[1], U. Schloetzer-Schrehardt[2], G.O.H. Naumann[2], B. Rautenstrauss[1]*. 1) Inst Human Genetics, Friedrich-Alexander Univ, Erlangen, Bavaria, Germany; 2) Department of Ophthalmology, FAU Erlangen-Nuremberg, Bavaria, Germany.

Primary open-angle glaucoma (POAG) is a clinically and genetically heterogeneous disease. Juvenile open-angle glaucoma (JOAG) is an early-onset form and is linked to the GLC1A locus on chromosome 1q24.3-24.2. For several GLC1A-linked families and sporadic cases mutations were found in the trabecular-meshwork inducible glucocorticoid response (TIGR) gene, synonymous Myocilin (MYOC. A mutation screening in 530 German POAG patients resulted in 6 % being carriers of mutations in exon 3 of the MYOC gene. In JOAG families 3 missense mutations were found. A predominant mutation is the Gln368Stop, predicting a protein truncated by 136 aminoacids. Surprisingly this mutation was found not only in 5 POAG patients, but also in 1 patient with normal tension glaucoma and in several healthy individuals. Taken together this results suggests that MYOC may play a crucial role in some but not all cases of POAG and that other, additional pathological factors are implicated in POAG. As different groups reported GLC1A-linked families without mutations in the MYOC gene there still might be another yet unknown gene in the GLC1A locus involved in the pathogenesis of POAG. One candidate in our study is the Cyclooxygenase 2 (COX-2) gene, located in close vicinity to MYOC. Cyclooxygenase is a key enzyme in the biosynthesis of prostaglandins. Prostaglandins appear to play an important role in the regulation of intraocular pressure. COX-2 is a steroid-regulated enzyme. It was found in reduced amounts in eyes of patients suffering from POAG. A mutation screening in the COX-2 gene of 50 patients with POAG revealed two different mutations in intron 6. One of them, suggesting a potential activation of a cryptic branching site, is cosegregating with a Pro370Leu MYOC mutation in a JOAG family. The second intronic mutation in the splice acceptor site was found in a single case. These intronic mutations may lead to a decresead splicing efficiency and subsequently to a decreased amount of COX-2 enzyme.

Program Nr: 2732 from the 1999 ASHG Annual Meeting

Localization of the putative transcription factor FMR2 in the mammalian brain. *W.J. Miller, J.A. Skinner, K.E. Davies.* Department of Human Anatomy and Genetics, University of Oxford, Oxford, OX1 3QX, United Kingdom.

The cytogenetic expression of the folate sensitive fragile site, FRAXE, on Xq28 of the human X chromosome is associated with a mild form of mental handicap characterised by learning deficits, including speech delay, reading and writing problems. The chromosome fragility is caused by the expansion of a CCG trinucleotide repeat array in the 5' region of the FMR2 (fragile X mental retardation 2) gene. The FMR2 messenger RNA is highly expressed in the placenta and brain but is absent from adult heart, lung, kidney, pancreas and skeletal muscle. FMR2 encodes a large protein of 1311 amino acids and is a member of a gene family encoding proline-serine rich proteins which have properties of nuclear transcription factors. In order to characterise the function and expression of the FMR2 protein, two human fusion proteins N-terminal (A) and middle (B), have been expressed using the pET32a expression system (Novagen) in *E.coli* . These fusion proteins were used to raise polyclonal antibodies in rabbits. Both antibodies detect a band of approximately 141 kDa on western blots of total mouse brain extract which correspond to the size predicted from the open reading frame of FMR2. FMR2 antibodies have been used for immunofluorescence experiments on mouse brain cryosections. Preliminary results demonstrate that FMR2 protein is localised in the neocortex, Purkinje cells of the cerebellum and the granular cell layer of the hippocampus. FMR2 staining appears to co-localise with the nuclear stain DAPI which strengthens the hypothesis that it acts as a transcription factor. In addition there appears to be a sub-set of neurones that contain the FMR2 protein. To date very little is known about the function of FMR2. Since the FMR2 protein has now been shown for the first time localised to the mammalian hippocampus and other brain structures involved with cognitive function we can now begin to investigate the possible function of the FMR2 protein in learning and memory.

Program Nr: 2733 from the 1999 ASHG Annual Meeting

A three generation-family with anterior segment mesenchymal dysgenesis and mutation in a novel homeobox-containing gene, VSX1. *H.A. Mintz-Hittner[1], E.V. Semina[2], J.C. Murray[2,3]*. 1) Dept Ophthalmology, Univ Texas-Houston Medical Sch, Houston, TX; 2) Dept Pediatrics, Univ Iowa, Iowa City, IA; 3) Dept Biol Sciences, Univ Iowa, Iowa City, IA.

The propositus of a three-generation family with autosomal-dominant anterior segment mesenchymal dysgenesis was found to have Axenfeld anomaly, bilaterally. The left eye had congenital and progressive corneal opacity and glaucoma. The iris of the right eye had neither collarette nor crypts by slit lamp biomicroscopy. There were no iris vessels identified by indocyanine green angiography. The optic nerve had peripapillary thinning and remnants of the embryonic hyaloid vascular system on its surface. Thus, the embryonic hyaloid vascular system appear to be affected anteriorly and posteriorly. In addition to the ocular findings, the propositus had a broad flat nasal bridge, abnormal pinnae and hearing loss, bilaterally. Other affected family members had similar but less severe findings.DNA samples from affected individuals from this family were analyzed for mutations in PITX2, PITX3 and FKHL7 genes, previously shown to be responsible for similar human phenotypes, and also a novel ocular homeodomain- containing transcription factor gene, VSX1. Two missense mutations in the VSX1 gene were identified: one predicts an arginine-to-serine change (R131S) in the N-terminal region of the encoded protein and another an alanine-to-serine substitution (A256S) in the highly conserved CVC-domain of the protein, located immediately C-terminal to the homeodomain. Function of the CVC-domain is unknown, but its proximity to the homeodomain is consistent with its participation in DNA-binding. Both of the mutations appear to reside on the same chromosome and co-segregated with the phenotype in this three-generation family. The R131S variant was found in approximately one-half percent of controls, while the A256S mutation was not found in any of 400 control chromosomes. The protein encoded by the VSX1 gene is most highly homologous to the zebrafish vsx1, goldfish vsx2 and mouse chx10 proteins, previously shown to play an important role in ocular development in different species. The VSX1 gene was mapped to 20p11-q11.

Program Nr: 2734 from the 1999 ASHG Annual Meeting

Mutations in the gene for alpha-cardiac actin in familial hypertrophic cardiomyopathy. *J. Mogensen[1,3], H. Egeblad[1], I.C. Klausen[1], T.A. Kruse[2], N. Gregersen[3], P.S. Andersen[5], A. Perrot[4], O. Havndrup[5], M. Christiansen[5], H. Bundgaard[5], K.J. Osterziel[4], P. Bross[3], A.K. Pedersen[1], A.D. Borglum[2]*. 1) Department of Cardiology, Skejby University Hospital, Aarhus, Denmark; 2) Institute of Human Genetics, University of Aarhus, Denmark; 3) Reseach Unit for Molecular Medicine, Skejby University Hospital, Denmark; 4) Charite/Frantz-Volhart-Klinik, Berlin, Germany; 5) Department of Cardiology, Rigshospitalet, Denmark.

Background: Familial hypertrophic cardiomyopathy (FHC) is an autosomal dominantly inherited disease caused by mutations in genes encoding sarcomeric polypeptides. We have recently reported that the alpha-cardiac actin gene (ACTC) is the 8th FHC gene. An Ala295Ser mutation was localized in exon 5 close to two missense mutations described to cause the inherited form of idiopathic dilated cardiomyopathy (IDC). Thus ACTC is the first sarcomeric gene described in which mutations are responsible for two different cardiomyopathies. Purpose: In order to estimate the frequency of ACTC mutations in FHC we are currently screening 70 Danish FHC families and 150 German probands for ACTC mutations. Methods: Individuals were considered to have FHC if they had hypertrophy of the myocardium (>13mm), hypertrophy in the ECG or Q-waves/major ST-segment changes in at least 2 ECG leads. The genetic investigations included mutation analyses by direct sequencing or SSCP. Results: So far 40% of the patients have been analyzed and a Met305Leu mutation has been identified in exon 5 of a German proband having apical hypertrophy and bradyarrythmia. The Met305Leu mutation identified is localized close to a myosin binding site and within a short distance of the bound actin ATP. Both Ala295 and Met305 are situated on the same face of domain 3 of the actin molecule. Conclusion: On basis of analysis of 88 patients the frequency of ACTC mutations in FHC seems to be above 2%. The fact that the Met305Leu mutation as well as the Ala295Ser mutation are localized in proximity to myosin binding sites supports the hypothesis that ACTC mutations affecting sarcomere contraction lead to FHC while mutations affecting force transmission from the sarcomere to the surrounding syncytium lead to IDC.

Program Nr: 2735 from the 1999 ASHG Annual Meeting

Analysis of SMN binding partners using a yeast two-hybrid system. *P. Mohaghegh, L. Campbell, K. Hunter, K.E. Davies.* Dept. Human Anatomy & Genetics, Univ Oxford, Oxford, England.

Childhood onset Spinal Muscular Atrophy (SMA) is autosomal recessive disorder, which maps to 5q13. It is caused by mutations in the survival motor neuron (SMN) gene that is a ubiquitously expressed gene, which has been implicated in RNA processing. However, how the function of SMN relates to the specific motor neuron death remains unsolved.

We have used a yeast two-hybrid assay to look for binding partners of SMN in mouse brain. SMN has previously been shown via yeast-2-hybrid system to interact with SIP1 and Bcl-2 proteins using a HeLa cDNA library (Liu & Dreyfuss 1996) and a thymus cDNA library (Iwahashi et.al 1997) respectively.

We have isolated several novel binding partners as well as SMN and SIP1. Database searches with the new binding partners revealed one of these to be part of cytosolic transport factors. We have made several SMN deletion constructs and have established via yeast two-hybrid approach which domain of SMN interacts with this cytosolic protein complex. We furthermore have determined this interaction via co-immunoprecipitation and co-localisation experiments. The relevance of this to the disease pathology is currently being explored.

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Program Nr: 2736 from the 1999 ASHG Annual Meeting

Infantile GM1-gangliosidosis: new mutations in the b**galactosidase gene identified in two patients with cardiomyopathy.** *A. Morrone[1], T. Bardelli[1], A. Caciotti[1], M.A. Donati[1], G. De Gregorio[1], A. d'Azzo[2], E. Zammarchi[1].*
1) Dept. Pediatrics, Univ Florence, Florence, Florence, Italy; 2) Dept. of Genetics, St. Jude Hospital, Memphis, USA.

Infantile GM1-gangliosidosis is a rare lysosomal storage disorder with autosomal recessive inheritance. The disease is caused by primary deficiency of acid b-Galactosidase and it is clinically characterised by early psychomotor delay, severe and progressive neurological involvement. Death usually occurs within two years. The human b-galactosidase (Hb-Gal) gene gives rise to two alternatively spliced mRNAs: a major transcript (Hb-Gal long) of 2,5 kb that encodes the classical lysosomal form of the enzyme, and a minor transcript (Hb-Gal short) of 2.0 kb encoding Elastin Binding Protein (EBP). Here we report the identification of three new b-galactosidase gene mutations in total RNA and DNA preparations of an Italian and an American patients affected by infantile GM1 gangliosidosis with cardiomyopathy. In the Italian patient an out-of-frame Hb-Gal transcript with 154 bp insertion in E10/E11 was detected in combination with the previously reported stop codon mutation W92X. The aberrant Hb-Gal transcript, has also been confirmed in his father's mRNA. These genetic lesions give rise to aberrant mRNAs with a complete lack of function of both the lysosomal protein and EBP. In the American patient,two new transitions,C252T and C1356T,have been identified that results in the aminoacid substitutions R68W and L436F. In addition to these mutations,the previously reported R201C substitution was also identified in this patient. The R68W has been confirmed in the father's genomic DNA. Both L436F and R201C have been identified in the mother and sister. The mutation R201C, mapping in a CpG island of a region encoding only for the lysosomal enzyme, correlates with juvenile GM1-gangliosidosis phenotype. Therefore the severe clinical manifestations must be attributed with R68W/L436F. These aminoacid changes are present in the Hb-Gal premRNA region common between Hb-Gal long and Hb-Gal short, consequently both proteins are affected by the mutations.

Program Nr: 2737 from the 1999 ASHG Annual Meeting

GENETIC STUDY OF A LARGE ADPKD AND PSYCHOSIS TUNISIAN FAMILY. *R. M'RAD[1], R. Zemni[1], F. Maazoul[1], F. El younsi[2], N. Smaoui[1], H. Ben Maiz[2], H. Chaabouni[1]*. 1) genetique, faculte de medecine de Tunis, Tunis, tunisia; 2) Service de nephrologie hopital Charles Nicolle.

Autosomal dominant polycystic kidney disease (ADPKD) is a genetically heterogeneous disorder. At least, two distinct forms of ADPKD are well defined. In ~86% of affected families, a gene defect localised to 16p13.3 was responsible for ADPKD while a second locus has been localised to 4q13.q23 as candidate gene for the disease in the remaining families. Here, we report a large ADPKD Tunisian family with 15 members; 12 (9 females, 3 males) of whom show clearly ultrasonografic scanning signs of ADPKD. 3 (2 females, 1 male) of the 12 shows signs of ADPKD associated with psychosis. To establish Linkage status in this family, we mainly used 3 highly polymorphic DNA markers, 3'HVR, KG8 and SM6 flanking PKD1 gene on 16p13.3 and 2 highly polymorphic DNA markers, 046 and 224, flanking the PKD2 gene. By means of molecular haplotypes identification, we show that this family is unlinked neither to PKD1 nor PKD2. Our data support a theory of more than 2 loci being responsible for ADPKD.

Program Nr: 2738 from the 1999 ASHG Annual Meeting

Molecular analysis and clinical characterization of nonsyndromic, autosomal recessive (DFNB) hearing loss. *A. Murgia[1], R. Polli[1], M. Martella[1], C. Vinanzi[1], E. Leonardi[1], E. Orzan[2].* 1) Dept Pediatrics, Univ Padua, Padua, Italy; 2) Service of Audiology, Univ Padua, Italy.

In industrialized countries approximately 1/1000 children is affected by preverbal hearing loss. In the majority of the cases this is a genetic, nonsyndromic, autosomal recessive condition (DFNB). It has been demonstrated that the single most common form of congenital preverbal nonsindromic deafness is due to mutations of the Cx26, GJB2 gene, which accounts for at least 50% of the cases (DFNB1), with a carrier frequency of about 1/30 in the mediterranean area. Nevertheless several other DFNB loci have been mapped and five of the corresponding disease-genes have been identified: MYO7A (DFNB2), MYO15 (DFNB3), PDS (DFNB4), OTOF (DFNB9), TECTA (DFNB21). With the aim of a clinical and molecular characterization of these forms of hearing loss, we have carefully selected, on the bases of a protocol that minimizes the possible inclusion of acquired deficits, a population of more than 200 individuals, aged between 3 and 35 years, affected by sensorineural, nonsindromic, autosomal recessive or sporadic hearing impairment. Molecular analysis of the Cx26 gene has allowed to identify mutations in 49% of our cases. The hearing loss in these latter individuals has been found to be of variable severity even within families, always preverbal, possibly not always congenital, and never progressive. No de-novo mutations have been found. The M34T Cx26 variant has not been found to act as a dominant mutation in three families of our series. The group of clinically selected subjects, who resulted negative for Cx26 mutations, represent now a particularly interesting population for the study of the role of other DFNB genes. We are characterizing, by PCR SSCP/CSGE analysis and direct sequencing, the exons of myosin 7A, myosin 15, pendrin, otoferlin and alpha-tectorin, that have been found mutated in cases of autosomal recessive nonsyndromic deafness. This analysis may contribute important information for a better understanding of the molecular bases and the clinical characteristics of these heterogeneous conditions.

Program Nr: 2739 from the 1999 ASHG Annual Meeting

Lysinuric protein intolerance (LPI) gene SLC7A7: Fine mapping and spectrum of mutations. *J. Mykkanen[1], D. Torrents[2], M. Pineda[2], A. Zorzano[2], V. Nunes[3], M.E. Yoldi[4], K. Huoponen[1], A. Reinikainen[1], O. Simell[1], M.-L. Savontaus[1], M. Palacín[2], P. Aula[5].* 1) Dept Medical Genetics, Univ Turku, Turku, Finland; 2) Dept Biochemistry and Molecular Biology, Univ Barcelona, Spain; 3) Dept Molecular Genetics (IRO), Hospital Duran i Raynalds, Barcelona, Spain; 4) Dept Neuropediatry, Hospital Virgen del camino, Pamplona, Spain; 5) Dept Medical Genetics, Univ Helsinki, Finland.

Lysinuric protein intolerance (LPI; MIM 222700) is an autosomal recessively inherited metabolic disorder characterised by basolateral plasma membrane transport defect of cationic amino acids presenting growth failure, hepatosplenomegaly, osteoporosis, postprandial hyperammonemia and aversion to dietary protein. We have previously identified a human cDNA, encoding y^+LAT-1 (y^+L Amino Acid Transporter-1), as the LPI gene SLC7A7. y^+LAT-1 co-expresses and induces y^+L transport activity with the surface antigen 4F2hc in *Xenopus* oocytes. In Finland LPI is more prevalent (incidence 1:60000) than in other parts of the world and all the Finnish patients studied share the same founder mutation.

In order to perform fine mapping of SLC7A7 gene, a PAC library was screened. PACs found were hybridised on DNA fibers together with y^+LAT-1 cDNA and a control PAC with known insert size. Distance measurement of 21 fibers showed SLC7A7 gene to be located 250 kb distal from T-cell receptor a/d intragenic microsatellite TCRA. The non-Finnish patients with Caucasian and Arabic ethnic background were screened for mutations at cDNA and/or genomic DNA level using RT-PCR of full y^+LAT-1 ORF, genomic PCR amplification of exons and automated sequencing. The genomic mutations found comprise nonsense, missense and splice site mutations, small deletions and a tandem duplication. A homozygous nonsense mutation 1012 G®A was found to be common in 4 LPI patients of Arabic ethnic background. The missense mutation 447 G®T found in Estonian and Latvian LPI patients changes fully conserved amino acid in this protein family of 4F2hc subunits reducing totally the transport activity of dibasic amino acids arginine and leucine when injected into *Xenopus* oocytes.

Program Nr: 2740 from the 1999 ASHG Annual Meeting

Homomeric channels of P932L mutations in muscle chloride channel gene (hClC-1) is associated with a severe myotonic myopathy. *S. Nagamitsu[1,2], T. Matsuura[1,2], M. Khajavi[1,2], R. Armstrong[1], C. Gooch[1], T. Ashizawa[1,2].* 1) Department of Neurology, Baylor College of Medicine, Houston, TX; 2) Neurology service Veterans Affairs Medical Center, Houston, TX.

Chloride channelopathies (dominant myotonia congenita, Becker myotonia) are nonprogressive, nondystrophic skeletal muscle disorder caused by a variety of mutations in the gene encoding the muscle chloride channel (hClC-1). So far over forty mutations have been identified in hCLC-1 gene. One-third of the identified mutations are nonsense mutations or frameshift mutations. These mutations result in a truncation of the predicted protein product. In diseases caused by nonsense or frameshift mutations, the mutant mRNA may be selectively degraded, thus, the organism is supposed to be protected from deleterious truncated protein. The two members of the family showed a unique myotonic myopathy with early development of proximal muscle hypertrophy, late onset of generalized progressive muscle weakness and predominantly distal atrophy with joint contracture. Clinically, the unique phenotype of this myotonic disorder clearly distinguishes this familys diseases from other myotonic disorders. We identified two novel mutations comprising a compound heterozygous state in this family : (1) a base (G) insertion in exon 7 generating early stop codon (fs289X) in the putative transmembrane domain D5, and (2) a C-to-T substitution in exon 23 resulting in a missense mutation (P932L). RT-PCR analyses of total RNA were extracted from one patient's affected muscle showed the expression of only P932L mutant mRNA. The fs289X mutant mRNA was undetectable, suggesting that it is degraded rapidly after transcription.

We propose a hypothesis that fs289X is a null mutation, rendering the patients with the compound heterozygous genotype of fs289X/P932L to exclusively express P932L homomeric channels that cause the severe phenotype.

Program Nr: 2741 from the 1999 ASHG Annual Meeting

A donor splice site mutation in intron 4 of the fibrinogen alpha-chain gene (*FGA*) is the common defect in congenital afibrinogenemia. *M. Neerman-Arbez[1], P. de Moerloose[2], A. Honsberger[1], C. Rossier[1], E.G.D. Tuddenham[3], S.E. Antonarakis[1], M.A. Morris[1]*. 1) Division of Medical Genetics, University Medical Centre and University Hospital, Geneva, Switzerland; 2) Division of Angiology and Hemostasis,University Hospital, Geneva, Switzerland; 3) Haemostasis Research Group, MRC Clinical Services Centre, Imperial College School of Medicine, London, U.K.

Congenital afibrinogenemia is a rare, autosomal recessive disorder characterised by the complete absence of detectable fibrinogen. We previously identified the first causative mutations in a non-consanguineous Swiss family; the four affected male individuals have homozygous deletions of approximately 11 kb of the fibrinogen alpha (*FGA*) gene on 4q28-4q31. Haplotype data imply that these deletions occurred separately, on distinct ancestral chromosomes, suggesting that the *FGA* region of the fibrinogen cluster may be susceptible to deletion by a common mechanism. The deletion junctions were initially mapped to the unsequenced *FGA-FGB* intergenic region by PCR and Southern blot analysis. We subsequently cloned this region following long-range PCR, and sequenced the deletion junctions of all three mutated chromosomes. We found that all the deletion junctions were identical, and probably resulted from non-homologous recombination involving two 7 bp direct repeats, AACTTTT, situated in *FGA* intron 1 and in the *FGA-FGB* intergenic sequence. We collected 13 additional unrelated patients with congenital afibrinogenemia from France, U.K. and Belgium in order to identify the causative mutations in affected individuals, and to determine the prevalence of the 11kb *FGA* deletion by sequence analysis and Southern blotting. No further patients were found to be carriers of the deletion. A second mutation, however, in the invariant GT dinucleotide (GT>TT) at the donor splice site of *FGA* intron 4, accounted for 12 out of 20 mutant alleles (60%).Two frameshift mutations were also found in compound heterozygosity in one further affected individual. Interestingly, all are early truncating mutations in *FGA* although mutations in all three fibrinogen genes, *FGG*, *FGA* and *FGB* are predicted to cause congenital afibrinogenemia.

Program Nr: 2742 from the 1999 ASHG Annual Meeting

Usher type IIa: Spectrum of Mutations in Danish and Norwegian Patients. *O. Nilssen[1], B. Dreyer[1], T. Rosenberg[2], M.D. Weston[3], W.J. Kimberling[3], L. Tranebjærg[1].* 1) Dept Med Genetics, Univ Hospital, Tromsø, Norway; 2) National Eye Clinic for Visually Impaired, Hellerup, Denmark; 3) Dept of Genetics, Boystown National Research Hospital, Omaha, NE.

Usher syndrome type II (MIM 276901/276905) is an autosomal recessive disorder characterized by hearing impairment from childhood and progressive retinitis pigmentosa from the late teens. Mutations in *USH2A*, located on 1q41 were recently shown to be responsible for Usher type IIa. The *USH2A* gene encodes a protein of 1546 amino acids that contains laminin type epidermal growth factor (LE)- and fibronectin type III like motifs. However, the exact function of USH2A is not known.

We have investigated the molecular pathology of Usher type II by screening the *USH2A* gene for mutations in a panel of 31 unrelated patients from Denmark and Norway. Mutations were detected by PCR-based exon amplification and direct, automated DNA sequencing.

Besides from the frequent 2299delG mutation, which accounted for 48% of the disease alleles, a heterogeneous spectrum of mutations was identified. Sixteen novel, putative disease-causing mutations were detected, of which 12 were private and 4 occurred in more than one family. The disease-causing mutations were scattered throughout the gene and included 7 missense mutations, 6 nonsense mutations, 2 small deletions and 1 small insertion. In addition, 6 polymorphic sites were identified.

All missense mutations resulted in major alterations in the properties of the corresponding amino acid side chains. Four missense mutations affected the N-terminal domain whereas 3 missense mutations affected the laminin type epidermal growth factor like (LE) domain of the USH2A protein. The structural consequences of the LE mutations are discussed in relation to the 3-dimensional structure of LE-domains.

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Program Nr: 2743 from the 1999 ASHG Annual Meeting

Spectrum of minor lesion NF1 gene mutations in 521 unrelated German neurofibromatosis type 1 patients*NF1*.

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In excess of 500 unrelated neurofibromatosis type 1 patients have been screened for mutations in the NF1 gene. For each patient the whole coding sequence and all splice sites were studied for aberrations either by the protein truncation test (PTT), temperature gradient gel electrophoresis (TGGE) of genomic PCR products, or mostly by direct genomic sequencing (DGS) of all individual exons. A total of 301 sequence variants including 278 bona fide pathogenic mutations were identified. As many as 216 of the genuine mutations, among them 179 different ones, can be considered novel when referring to the most recent review in the field (Upadhyaya and Cooper, 1998). Mutation detection efficiencies of the different screening methods were very similar to each other, 47.1 % for the PTT, 53.7 % for TGGE, and 54.9 % for DGS. Some 224 mutations (80.2 %) yielded directly or indirectly premature termination codons. These mutations showed a very even distribution over the whole gene from exon 1 to exon 47. Of all sequence variants determined in our study, less than 20 % represent C to T or G to A transitions within a CpG dinucleotide and only six different mutations do also occur in NF1 pseudogenes with five of them being typical C to T transitions in a CpG. Thus, neither frequent deamination of 5-methylcytosines nor interchromosomal gene conversion may account for the high mutation rate of the NF1 gene. As opposed to the truncating mutations, the 28 (10.1 %) missense or single amino acid deletion mutations identified clustered in two distinct regions, the GRD and an upstream gene segment forming a so-called cysteine/serine-rich domain. Coincidence of mutated amino acids and those conserved between human and Drosophila strongly suggests significant functional relevance of this region.*DrosophilaNF1*.

Program Nr: 2744 from the 1999 ASHG Annual Meeting

Genetic heterogeneity in hereditary and autonomic sensory neuropathy type 4 (HSAN4). *C. Oddoux[1], J. Wang[1], C.M. Clayton[1], M. Hilz[1], R. Cilio[2], E. Bertini[2], C. Mayaan[3], A. Blumenfeld[3], F. Axelrod[1], H.. Ostrer[1].* 1) New York University Medical Center, New York, NY; 2) Ospedale Bambino Gesu, Rome, Italy; 3) Hadassah Hospital, Jerusalem, Israel.

HSAN4 is an autosomal recessive disorder characterized by insensitivity to pain and anhydrosis, and is usually associated with self mutilation, mental retardation, and absence of histamine-induced axon flare. Previously, mutations in the tyrosine kinase domain (Trk) of the high affinity nerve growth factor receptor (NTRK1) have been observed in individuals with HSAN4 (Indo, et al., 1996 Nat Genet 13:485). To determine whether mutations in other regions of the NTRK1 gene or other genes in the NGF signal transduction pathway cause HSAN4, we studied 8 families with affected children; three from northern Italy, three from southern Italy, one Greek and one Bedouin. With the exception of the northern Italian families all were consanguineous. Haplotypes of the NTRK1 flanking and intragenic markers D1S2624, D1S506, and D1S116, demonstrated the absence of a founder mutation among the Italian patients and were compatible with linkage to NTRK1 for all of the families. Direct sequence analysis of the NTRK1 gene revealed that two individuals had homozygous mutations within the Trk domain, one of which was a complex allele with two sequence changes. Another patient with a particularly mild phenotype (some residual pain sensation and sweating, no self mutilation, and normal intelligence) had two different mutations distal to the Trk domain in exon 17, suggesting incomplete impairment of signal transduction. This study suggests that most cases of HSAN4 are caused by mutations in NTRK1 and is the first to demonstrate that mutations outside the Trk domain can give rise to either comparable or milder disease.

Program Nr: 2745 from the 1999 ASHG Annual Meeting

Molecular analysis of Greig cephalopolysyndactyly syndrome shows a high frequency of deletions. *I.M. Olivos-Glander[1], J. Blancato[2], J. Meck[3], L.G. Biesecker[1]*. 1) Genetic Disease Research, NIH/NHGRI, Bethesda, MD; 2) Institute of Molecular Human Genetics, Georgetown University, Washington, D.C; 3) Department of Obstetrics and Gynecology, Georgetown University, Washington, D.C.

We have ascertained a cohort of 23 families with one or more persons affected by Greig cephalopolysyndactyly syndrome (GCPS). Diagnostic criteria include preaxial polysyndactyly of the feet with either macrocephaly or hypertelorism. These families consist of 19 sporadic cases and four familial cases. We are developing a diagnostic protocol to systematically assess the wide range of genetic alterations that are capable of generating this phenotype. The protocol begins with high resolution karyotyping with attention directed to 7p13, the locus of the Gli3 gene that is known to be haploinsufficient in many GCPS patients. Next, to assess for submicroscopic deletions, we use both microsatellite analysis and FISH analysis using cosmids and BACs. Biallelic cDNA expression assays will be performed next to select patients for sequencing or Southern analysis. Patients who express both alleles will be sequenced and those who do not will be analyzed by a series of Southern analyses for this large (~290 kb) gene.

We have so far identified that at least 7 of the 23 affected probands have gene deletions either by microsatellite analysis, Giemsa banding or FISH analysis. Future work will include determining the extent of the deletions in those patients identified with a deletion and performing the biallelic expression assay in those who are not deleted in GCPS. The high frequency of deletions in GCPS patients precludes sequencing as a logical first step and we conclude that an integrated diagnostic scheme would be useful in this disorder.

Program Nr: 2746 from the 1999 ASHG Annual Meeting

Towards mouse models for Parkinson's disease: humanizing the a-**synuclein locus.** *B.M. Orrison, D.E. Cabin, S. Gispert, A. Chen, L. Garrett, R.L. Nussbaum.* Genetic Disease Research Br, NHGRI/NIH, Bethesda, MD.

Mutations in the a-synuclein gene (*SNCA*), which encodes a major component of Lewy bodies, were the first to be associated with familial forms of Parkinson's disease. The mouse homolog was mapped to mouse chromosome 6 (MMU6) at 26.8 cM on the Jackson Laboratory BSS backcross panel. This establishes a new region of shared synteny between MMU6 and human chromosome 4, to which *SNCA* was previously mapped. No possible candidate neurological phenotypes map to that region of MMU6. As the first step in developing animal models for the disease, the a-synuclein locus has been knocked out in the mouse by replacing the second and third coding exons with the neomycin resistance gene. Mice homozygous for the deletion produce no a-synuclein detectable by protein blotting, are viable, and have no obvious phenotypes. Matched +/+ and -/- pairs are being bred for behavioral testing, and mutant animals are being aged to determine if any phenotypes may develop late in life. The -/- animals are also being bred to transgenic mice carrying both the human wild type and A53T mutant versions of a-synuclein. The wild type human gene is carried on a PAC and is under the control of its endogenous promoter; the A53T mutant transgenic animals carry a cDNA construct driven by the prion promoter. The PAC transgenic animals carry about 25 copies of the transgene, and the A53T mutant cDNA is present at more than 10 copies. Currently intercrosses of animals carrying both transgenes on a +/- a-synuclein background are in progress. Animals that carry both these transgenes on the mouse null background will mimic the situation seen in a familial form of Parkinson's disease.

Program Nr: 2747 from the 1999 ASHG Annual Meeting

Multiorgan autonomic dysfunction in mice lacking the b2 and the b4 subunits of neuronal nicotinic acetylcholine receptors. *A. Orr-Urtreger[1], W. Xu[2], F. Nigro[3], S.E. Gelber[5], C.B. Sutcliff[2], L. Goldberg[2], R.A. Lewis[4], D. Armstrong[4], C-N. Ou[3], J.W. Patrick[3], L.W. Role[5], M. De Biasi[3], A.L. Beaudet[2].* 1) Genetics Inst, Tel-Aviv Sourasky Medical Ctr, Tel Aviv, Israel; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas; 3) Division of Neuroscience, and; 4) Departments of Ophthalmology and Pathology, Baylor College of Medicine, Houston, Texas; 5) Department of Anatomy and Cell Biology and Center for Neurobiology and Behavior, Columbia University, New York, NY.

We have generated mice with null mutations in the b2 and the b4 subunits of the neuronal nicotinic acetylcholine receptor (nAChRs) genes and created double null mice (b2-/-b4-/-) by mating. The b2-/- and the b4-/- single mutant mice grow to adult life with no visible phenotypic abnormalities. The b2-/-b4-/- mutants survive to birth but have impaired growth and increased perinatal mortality. They also present enlarged bladders with dribbling urination and develop urinary infection and bladder stones. The ocular pupils are widely dilated and do not contract in response to light. Histological studies revealed no significant abnormalities of brain and peripheral tissues except for hyperplasia in the bladder mucosa of the b4-/- and the b2-/-b4-/- mutants. Bladder strips from b2-/-b4-/- mice did not respond to nicotine but contracted when stimulated with a muscarinic agonist or electric field stimulation. Bladder strips from b4 mutants did not respond to nicotine despite the absence of major bladder dysfunction in vivo. Acetylcholine-activated whole-cell currents were absent in superior cervical ganglion neurons from b2-/-b4-/- mice and reduced in neurons from b4-/- mice. Our previous results with the a3-/- mutant mice ant the current data suggest that the a3 and the b4 subunits are the major components in autonomic nAChRs. The phenotype of the b2-/-b4-/- and a3 mice resembles the autosomal recessive megacystis-microcolon-hypoperistalsis syndrome in humans.

Program Nr: 2748 from the 1999 ASHG Annual Meeting

Methylation Studies in Facioscapulohumeral Muscular Dystrophy. *M.J. Osborn, D.N. Cooper, M. Upadhyaya.* Institute of Medical Genetics, Uni Wales College of Medicine, Cardiff, U.K.

Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant neuromuscular disorder characterized by progressive weakness of the facial, shoulder and upper arm muscles. The disorder has been mapped to 4q35 and is associated with the deletion of integral copies of a D4Z4 repeat. Although the D4Z4 repeat contains homeobox-like sequences, no transcript from this locus has been identified. It has been proposed that the underlying disease mechanism in FSHD is by deletion-mediated position effect variegation (PEV). Thus D4Z4 deletions could place the proximally located FSHD gene closer to the heterochromatinizing influence of the 4q35 telomere. Assuming hypermethylation to be one consequence of heterochromatinization, establishing the methylation status of the 4q35 region in both FSHD and in control subjects might further define the chromosomal region affected by PEV. We have attempted to assess the methylation status of the 4q35 region in both FSHD patients and matched control individuals by means of methylation-sensitive restriction enzymes and bisulphite-modified genomic sequencing. Preliminary examination of the D4Z4 locus with methylation-sensitive enzymes revealed increased methylation of FspI and EagI sites within the 3.3kb repeat, whereas, by contrast, the SacII sites were to a large extent unmethylated. Analysis of the D4F104S1 region demonstrated a proximal HpaII site that exhibited 50% methylation which could be due to either the 4q or 10q homologue being methylated. Our preliminary results suggest that FSHD muscle DNA may be less methylated than normal muscle. Bisulphite sequencing of the promoter sequence of the FRG1 gene in normal and FSHD blood and muscle DNA failed to provide any evidence of methylation. We are currently investigating the methylation status of the sequences in the first D4Z4 repeat specific to 4q35 to determine if there is a difference between FSHD and normal samples or between the homologous chromosome 4 and 10 repeat loci.

Program Nr: 2749 from the 1999 ASHG Annual Meeting

Molecular cloning of the breakpoint junction in a family with nephronophthisis type 1. *E. Otto, R. Betz, C. Rensing, S. Schaetzle, T. Vetsi, T. Kuntzen, A. Imm, F. Hildebrandt.* Univ Childrens Hospital, Freiburg, Germany.

Juvenile nephronophthisis (NPH1) is an autosomal recessive kidney disorder, characterized by disrupted tubular basement membranes, cysts, and tubulo-interstitial nephropathy. The disease leads to end-stage renal failure at an average age of 13 years. The defective gene for NPH1 was identified as *NPHP1* by positional cloning at 2q13, however, its function is still unknown. Approximately 80% of all patients exhibited a 250 kb genomic deletion in the *NPHP1* region. In this study, we identified the proximal and distal deletion breakpoints in a family (F12) that bears a shorter 200 kb deletion. PCR deletion analysis revealed that this family has a unique proximal breakpoint within intron 2 of the *NPHP1* gene. By Southern blot restriction mapping we detected a 40 kb EcoRI junction fragment besides the normal 14 kb fragment. For breakpoint fine mapping, the entire 20.8 kb intron 2 and a 30 kb EcoRI fragment from the distal breakpoint region were sequenced using PAC clones. Across breakpoint PCR was carried out and yielded a 1,800 bp fragment which was sequenced directly. The proximal breakpoint was localized 360 bp downstream of the 5' end of a LINE 1 element of 6 kb. Besides this repetitive element, sequence analysis of the breakpoint genomic regions yielded no candidate sequence responsible for a deletion mechanism. The characterization of further breakpoint regions will be helpful to identify NPH1-patients with heterozygous deletions and may offer insights into deletion pathomechanisms.

Program Nr: 2750 from the 1999 ASHG Annual Meeting

Functional Analysis of *C.elegans* and *S.pombe* orthologues of the human Survival Motor Neuron (SMN) protein.

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Childhood onset Spinal Muscular Atrophy (SMA) is characterised by the loss of lower motor neurones and proximal muscle weakness. All three types of SMA result from defects of the gene for the Survival Motor Neurone (SMN) protein.

We have isolated and characterised SMN orthologues from both *Caenorhabditis elegans* (CeSMN) and *Schizosaccharomyces pombe* (*smn1+*). Both orthologues show a high degree of conversation with the human SMN at the amino acid level, particarlly in regions of the human protein with known function: at the 5` binding SIP1 and at the 3` binding the Sm proteins and including the YG box.

Utilising translational GFP tagged fusion proteins we have analysed cellular localisations of both orthologues and have observed phenotypes in both for over expression of the proteins. Using dsRNA interference we have reduced the CeSMN protein level, resulting in a spectrum of severe phenotypes including lethality. Similarly in *S.pombe* we have created targeted knockouts leading to lethality.

In *S.pombe* we have analysed missense mutations in specific amino acids of the protein that are absolutely conserved between yeast and man. The residues chosen are known to be SMA causing mutations seen in Type I patients. Over expression of the mutations in smn1 demonstrate a dominant negative effect on growth phenotype and a change in subcellular localisation of the protein, observed through GFP tagging. We have also created and analysed a new mutation in the yeast *smn1* gene to determine the phenotype and localisation of the protein. Analysis of human and mouse identical mutations and effects on SMN binding partners will be presented.

Program Nr: 2751 from the 1999 ASHG Annual Meeting

MEFV Mutation Analysis in Turkish Familial Mediterranean Fever Families. *T.H. Ozcelik[1,2], E. Erken[3], B. Cevher[2], G. Tuncman[2], L. Mesci[2], H. Ozer[3], R. Gunesacar[3], M. Ozturk[1,2].* 1) Dept Molecular Biol & Genetics, and; 2) BilGen, Bilkent University, Ankara Turkey; 3) Dept of Internal Medicine Cukurova University School of Medicine, Adana, Turkey.

Familial Mediterranean fever (MEFV) is an autosomal recessive disorder that frequently affects Turkish, North African Jewish, Arab, and Armenian populations. It is characterized by episodic attacks of inflammation in the peritoneum, synovium or pleura accompanied by fever, and skin rash. The FMF gene (MEFV) on chromosome 16 was identified recently. In addition the disorder was shown to be genetically heterogenous through linkage analysis in two Turkish families with clinical features of FMF. In order to determine the spectrum of MEFV gene mutations we collected blood samples from 42 unrelated Turkish FMF families predominantly from the Southern and Southeastern regions of the country. We screened the probands for four missense mutations in exon 10 by PCR amplification of genomic DNA and automated DNA sequencing. Distribution of the mutations in 84 independent FMF alleles is as follows: M680I (14), M694V (34), V694I (3), and V726A (13). Both FMF mutations were identified in 28 patients (66.6%), only one mutation was identified in eight patients (19%), and no mutation was found in six patients (14%). Based on these findings mutations in 76% of the carrier chromosomes were identified. However 85-93% of MEFV mutations were identified in other populations. The relatively low number of mutations in our study group may be due to genetic heterogeneity in FMF. In fact none of the families were selected through linkage analysis. In addition other MEFV mutations can be expected especially in the group of patients with only one mutation. We are now screening for E148Q mutation in the groups of patients with no mutation and only one mutation. We will further perform linkage analysis in mutation negative families. These results indicate that DNA-based diagnosis of FMF in the Turkish population may be helpful. However, determination of MEFV carrier frequency based on the above mentioned mutations appears to be early.

Program Nr: 2752 from the 1999 ASHG Annual Meeting

Connexin 26 mutations in Greek patients with prelingual deafness. *A. Pampanos[1], M. Grigoriadou[1], J. Economides[2], T. Iliadis[3], N. Voyiatzis[3], P. Neou[4], N. Apostolopoulos[4], T. Antoniadi[5], J. Petmezakis[5], V. Christophidou-Anastasiadou[5], K. Grønskov[5], K. Brøndum-Nielsen[5], J. Gyftodimou[1], A. Skevas[5], M.B. Petersen[1].* 1) Dept of Genetics, Inst Child Health, Athens, Greece; 2) "Aghia Sophia" Children's Hosp, Athens, Greece; 3) Aristotle Univ Thessaloniki, Thessaloniki, Greece; 4) "P. and A. Kyriakou" Children's Hosp, Athens, Greece; 5) Cx26 Consortium.

Mutations in the gene encoding the gap-junction protein connexin 26 (Cx26) on chromosome 13q11 have been shown as a major contributor to prelingual, sensorineural, non-syndromic recessive deafness in Caucasian populations. One specific mutation, a deletion of G in a sequence of six Gs (35delG), has accounted for the majority of the mutations detected in the Cx26 gene and is one of the most frequent disease mutations identified so far with highest carrier frequency in Southern European populations. We have previously detected a carrier frequency of the 35delG mutation of 3.5% in Greek people. In a collaboration with the major referral centers for prelingual deafness in Greece and Cyprus, patients were examined by an extensive questionnaire to exclude syndromic forms and environmental causes of deafness, and by allelle-specific PCR for the 35delG mutation. The 35delG mutation was detected in 33% of the alleles in 30 cases (27 sporadic, 3 familial): 9 homozygotes and 2 heterozygotes. Individuals non-homozygous for the 35delG mutation are further analysed by DGGE and dideoxy fingerprinting techniques and sequencing. Due to the marked preference of deaf persons for a deaf spouse, vertical transmission of deafness due to homozygosity of the 35delG mutation was observed in one family, not resembling the typical recessive pattern of inheritance. We conclude that all individuals with prelingual, sensorineural, non-syndromic deafness should be tested for the 35delG mutation, irrespective of pedigree pattern.

Program Nr: 2753 from the 1999 ASHG Annual Meeting

Estimate on the frequency of Connexin 26 mutations in the deaf population in the USA. *A. Pandya[1], R. Morell[3], K. Oelrich[2], K.S Arnos[2], X.J. Xia[1], X.Z. Liu[1], J. English[1], S.H. Blanton[1,4], A. Griffith[3], T. Friedman[3], W.E. Nance[1].* 1) Virginia Commomwealth University,Richmond, VA; 2) Gallaudet University, Washington D.C; 3) NIDCD, Rockville,MD; 4) University of Virginia, Charlottesville, VA.

Prelingual hearing loss affects 1:1000 children, at least half of which is genetic in etiology. The GJB2 gene encoding gap junction protein 2, also called Connexin 26 is one of a growing number of genes found to have mutations which can cause hearing loss. One particular mutation 35delG accounts for up to 50-80% of recessive deafness in the French, Spanish and Italian populations. A different mutation, 167delT, has a high prevalence in the Ashkenazi Jewish population with a carrier frequency of about 4%. At least 40 other GJB2 alleles associated with hearing loss have been reported. However, there is marked phenotypic variability in the degree of hearing impairment as well as progression. This, coupled with the relative ease of testing for mutations at this locus has raised important ethical and social issues. In order to address these issues and to estimate the frequency of different mutations at the GJB2 locus in the US population we have begun to ascertain deaf probands from both multiplex and simplex families through a national survey conducted by the Research Institute at Gallaudet University. Molecular analysis was performed by direct sequencing of the coding region for GJB2 locus. So far, we have observed six previously described and one new mutation. The new mutation is an Arg32Cys substitution in the first transmembrane domain in a compound heterozygote with congenital deafness. Connexin deafness accounted for 25% of the 92 US probands and at least 77% of the mutant alleles were 35delG. Audiometric data is available on 47 of these probands. Identification of the mutational spectrum at this locus will allow a careful genotype-phenotype correlation and provide more accurate estimates of the frequencies of various GJB2 alleles . This will enable provision of accurate diagnosis, prognosis and, counseling for language and speech as well as for future recurrence risk.

Program Nr: 2754 from the 1999 ASHG Annual Meeting

Screening of mutations in the Caveolin-3 gene in Brazilian limb-girdle muscular dystrophy patients. *F.de Paula[1], M. Vainzof[1,2], E.E. McNally[3], L.M. Kunkel[4], M. Zatz[1].* 1) Centro de Estudos do Genoma Humano, IB-USP, Sao Paulo, Brazil; 2) Dept. of Neurology, FMUSP; 3) Section of Cardiology, University of Chicago, Chicago, USA; 4) Division of Genetics and the Howard Hughes Medical Insitute, Childrens Hospital, Boston, USA.

Limb-girdle muscular dystrophy (LGMD) is a clinically and heterogeneous group of myopathies characterized by proximal muscular weakness and a variable clinical course. Eight autosomal recessive (AR) and 4 autosomal dominant (AD) forms have already been identified. Caveolin-3 (CAV3) is localized to the sarcolemma where it forms a complex with dystrophin and its associated glycoproteins. Mutations in the CAV3 gene, mapped at 3p25, with a reduction of more than 95% in the protein expression (Minetti et al.,1998), cause AD LGMD 1C. It has also been suggested that mutations in CAV3 might also cause AR LGMD (McNally et al., 1998).We have analyzed (through SSCP and sequencing of abnormal fragments) a total of 74 Brazilian LGMD patients for mutation in the CAV3 gene: 7 with AR , 53 isolated cases (22 women and 31 men) and 14 familial cases (6 with confirmed AD inheritance). Muscle protein analysis done in 49 patients excluded dystrophinopathies (in 49), sarcoglicanopathies (in 42) and calpainopathies (in 25). One female (previously reported) had a C71W change in one allele. Other missense mutations, in one of the alleles were detected in 3 isolated cases: G55S (in two males with mild course) and R125H (in a female with mild course). Analysis of the C71W change in 100 normal controls detected 1 case in heterozigous state and G55S in 2 cases (2%) also in heterozigozity. The R125H mutation was not found in 200 normal chromosomes but was present in 2 apparently unaffected sibs of the proband. IF analysis for caveolin-3 in muscle biopsies from patients with mutations G55S and R125H showed a strong positive sarcolemal pattern, suggesting no protein reduction. The possibility that these mutations in the CAV3 gene are interacting with other genes resulting in an abnormal phenotype cannot be ruled out. However, the present study suggests that the mutations in CAV3 gene, causing LGMD 1C, is rare in our population.
FAPESP, CNPq, PRONEX.

Program Nr: 2755 from the 1999 ASHG Annual Meeting

High carrier frequency of the hemochromatosis C282Y allele in factor V Leiden-positive patients. *J.E. Paulus-Thomas, J.E. Hudson, M. Matzinger.* MCP Hahnemann University, Pittsburgh, PA.

A significant association was found between heterozygosity for factor V Leiden mutation (R506Q) and the common hereditary hemochromatosis mutation (C282Y) in unselected patients referred for hypercoaguable testing (Xie et al, 1998). The frequency of C282Y carriers was 18.7% in factor V Leiden-positive cases compared to 3% in factor V-negative patients and 6% in control populations. A subsequent study of patients diagnosed with venous thromboembolism did not reveal an increased prevalence of the C282Y allele in factor V Leiden heterozygotes. Our investigation analyzed coinheritance of the C282Y and R506Q alleles in patients who were referred for factor V Leiden mutation analysis in comparison to unrelated Caucasian controls. Genomic DNA extracted from peripheral blood specimens was tested by PCR in combination with restriction digestion. In 50 patients who were referred for DNA testing and found to be factor V Leiden heterozygotes, 26% were also carriers of the C282Y allele and 74% were negative for C282Y. Two individuals homozygous for factor V Leiden mutation also possessed one copy of C282Y. In the random Caucasian group, only 6.4% were C282Y carriers and none of these individuals were homozygous C282Y. Our results support a significant association between R506Q and C282Y heterozygosity in patients referred for DNA testing and presumably at risk for hypercoagulation. Additional studies are required to identify the subgroup of these patients in which this association is highest and to unravel the biological relationship of these two alleles.

Program Nr: 2756 from the 1999 ASHG Annual Meeting

***APOE* haplotype segregating with Late-Onset Familial Alzheimer's Disease for 200 years.** *J. Perez-Tur[1], R. Crook[1], J. Hardy[1], D.S. Borgaonkar[1, 2]*. 1) Mayo Clinic Jacksonville. Jacksonville, FL; 2) Christiana Care Health System. Wilmington, DE.

Several genetic risk factors have been proposed to modulate the appearance of late-onset Alzheimer's disease (LOAD). Among these, only *APOE* is widely accepted to influence the risk for the disease. Moreover, recent evidence shows that genetic variability at this locus modifies the risk for the disease conferred by this isoform. We have previously described a large American pedigree affected by LOAD with a suggestive role for the e4 allele of *APOE*. Now, we present more detailed studies of this locus on chromosome 19. We genotyped the following microsatellite markers D19S178, APOC II, D19S908 and D19S918 and performed a parametric analysis of the data. In the modeling of the disease we took into account the influence of the e4 allele, that all definitely affected individuals were women and the existence of two consanguineous marriages in the pedigree. The maximum two-point LOD score observed in this pedigree was of 1.0 for marker D19S918 at q=0.0. This is in good agreement with the power calculations performed using the same parameters as in the linkage analysis. A haplotype was observed segregating with the disease but was also present in some clinically unaffected individuals. We sequenced the coding region of the gene looking for additional variation that could explain the apparently high risk conferred by possession of this haplotype in this family. In addition, we have determined the genotypes at the polymorphic sites -491, -427, -291 and E4P. No differences were observed between affected and unaffected individuals with regard to these polymorphisms. No new coding polymorphisms were observed. Finally, we also investigated the influence of other AD loci, *APP*, *PS1* and *PS2*, in this family but found no evidence of cosegregation of genotypes at any of these loci with the disease. Work supported by the Crystal Trust, the Rieffel Memorial Fund and the Mayo Foundation.

Program Nr: 2757 from the 1999 ASHG Annual Meeting

Mutational screening by DGGE in French PKD1 families. *R.A. PERRICHOT[1, 2], B. MERCIER[1], P. SIMON[3], B. WHEBE[4], J. CLEDES[2], C. FEREC[1]*. 1) Genetic laboratory, ETSBO - CHU, BREST, FRANCE; 2) Nephrology department, CHU, Brest - FRANCE; 3) Nephrology department, CH, Saint Brieuc - FRANCE; 4) Nephrology department, CH, Quimper - FRANCE.

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most commonly inherited renal disorders. Mutations in at least three different genes can lead to ADPKD. PKD1, the major locus, mapped to chromosome 16p13-3, accounts for approximately 85% of ADPKD cases. We screened 146 French unrelated patients for PKD1 mutations analysis by denaturating gradient gel electrophoresis (DGGE) approach. In the first step of the study, we have successfully identified novel mutations in the non duplicated region of the gene. The same approach has been used to analyse part of the duplicated area from exon 15 to 33 after a step of long range PCR followed by nested PCR for the analyse of each exon. This strategy allowed us to identify novel mutations that we report here. This is the first study using the DGGE approach for PKD1 mutation screening. From our data and others published in the literature the correlation of genotype-phenotype is not evident; variability of mutation and DNA variants exists widespread along the gene. In order to understand the molecular mechanisms underlying ADPKD we must screen for additional mutations along the entire PKD1 gene.

Program Nr: 2758 from the 1999 ASHG Annual Meeting

The SOX10 transcription factor: evaluation as a candidate gene for central and peripheral demyelination. *V. Pingault[1], N. Bondurand[1], C. Le Caignec[1], S. Tardieu[2], The European Network on Brain Dysmyelinating Diseases[3], N. Lemort[1], O. Dubourg[2], E. Le Guern[2], O. Boespflug-Tanguy[4], M. Goossens[1]*. 1) INSERM U468 et Laboratoire de Biochimie et Genetique Moleculaire, AP-HP, Hopital Henri Mondor, Creteil, France; 2) INSERM U289, Hopital de la Pitie-Salpetriere, Paris, France; 3) The European Network on Brain Dysmyelinating Diseases; 4) INSERM U384, Faculte de Medecine, Clermont-Ferrand, France.

Several *SOX* genes have already been shown to be transcription factors involved in the decision of important cell fates during development. The *Sox10* gene was first cloned from rat glial cells and shown to be expressed in neural crest cells during early development and in neural crest derivatives, including melanocytes and peripheral nervous system. In the central nervous system, the *Sox10* expression pattern suggests an expression in oligodendrocytes. The SOX10 factor acts as an autonomous transcription factor in humans, but also modulates the activity of others transcription factors involved in Schwann cells development, such as TST1/OCT6/SCIP, PAX3, Krox20/EGR2. We previously showed that heterozygous *SOX10* loss of function mutations result in Waardenburg-Hirschsprung disease, a condition characterized by enteric nervous system defect, hypopigmentation and deafness. Recently, another mutation was reported in a patient showing a central and peripheral demyelinating disease associated with Waardenburg-Hirschsprung syndrome. All these data suggest that *SOX10* could be a candidate gene for demyelinating disease. Here we report the results of mutation screening in 60 patients with Charcot-Marie-Tooth disease type 1, and in 87 patients expressing demyeliniting or hypomyelinating leukodystrophies (including a few patients with an associated peripheral neuropathy, spinal motor involvment, deafness or hypopigmentated areas of the skin).

Program Nr: 2759 from the 1999 ASHG Annual Meeting

Molecular basis of CD 36 deficiency in the African Bantu population. *S. pissard[1], K. Lee[2], F. Niels[1], R. Narwa[1], P. Bierling[2], B. Godeau[4], M. Goossens[1], F. Galacteros[3]*. 1) lab of Biochemistry and Genetics, Hop Henri Mondor, Creteil, France; 2) Transfusion Medicine Unit, Hop Henri Mondor, Creteil, France; 3) Centre de la Drepanocytose,Hop Henri Mondor, Creteil, France; 4) Internal Medicine Unit, hop Henri Mondor, Creteil.

CD 36 is a cell-surface glycoprotein (472 aa, 88 KD) expressed on a large set of cells including hematopoietic cells and "derivates" (platelets, monocytes, reticulocytes and young red cells), epithelial cells, adipocytes. The deficiency can be complicate by isoimmunization against platelets which results in transfusion refractoriness, post-transfusion purpura or neonate thrombocytopenia. About 5 to 10% of Asians lack platelet CD 36. This deficiency is very rare in the Caucasian population (less than 1%) and concerns 5 to 10 % of the whole African population . In a survey of the African population from sub-Saharan African countries, this frequency was found to be in the range of 10 to 20%. This unexpected high frequency is probably related to the role that CD 36 plays in the adhesion of malaria parasitized erythrocytes on epithelial cells. We have searched for the mutation responsible for CD 36 deficiency in 17 platelet-, CD36 - patients from this population using an RNA-based detection method (NIRCA). We did not find the P90S mutation described in the Japanese population, but we observed two recurrent mutations : 446 AA->G (exon4) in 10 patients and IVS 5, +2 T->C in 3 other patients. These two mutations lead to a frameshift and therefore inactivate the gene. We analysed the polymorphic microsatellite marker that lies in the 3rd intron of the CD 36 gene in the CD 36 - patients and in African Bantu newborns (control population). In the CD 36 - patients, the 46 AA->G mutation is always associated with the 196 allele, while the IVS 5, +2 TC mutation is associated with the 184 allele. The 196 allele seems to be significantly more frequent in CD 36- patients than in control population (27% to 11% $p<0.05$. since it is a rare allele, 184 do not show significant difference between CD 36- and control population (9 to 6%, ns). This could be an indication of founding mutations in Cd 36 deficiency in African Bantu population.

Program Nr: 2760 from the 1999 ASHG Annual Meeting

Functional analysis of a novel rhoGAP gene in Xp22.3. *S. Prakash[1], R. Paylor[1], B. Xu[1], H. Zoghbi[1,2].* 1) Baylor College Medicine; 2) Howard Hughes Medical Institute, Houston, TX.

Microphthalmia with Linear Skin defects (MLS) is an X-linked dominant, male lethal syndrome characterized by microphthalmia, aplastic skin and agenesis of the corpus callosum, and is caused by the deletion of a 500 Kb critical region in Xp22.3. We cloned a novel rho GTPase-activating protein (rhoGAP) gene named *ARHGAP6* from the MLS region. *ARHGAP6* contains 14 exons encoding a 975 amino acid protein with three putative SH3-binding domains. Exons 2-14 are deleted in all MLS patients. This led us to hypothesize that *ARHGAP6* may be responsible for some of the phenotypic features of MLS. RhoGAPs are regulators of cell migration and morphology through interactions with the actin cytoskeleton and another rhoGAP gene was recently implicated in nonsyndromic mental retardation. Two approaches were pursued to determine the role of *ARHGAP6* in the pathogenesis of MLS: gene targeting of the rhoGAP domain in embryonic stem cells and *in vitro* expression studies. Surprisingly, loss of the rhoGAP activity of *Arhgap6* does not cause any detectable phenotypic or behavioral abnormalities in the mutant animals. However, transient transfection studies suggest that the rhoGAP domain may be dispensible for some functions of the protein. Transfected HeLa and COS7 cells expressing ARHGAP6 retract from the growth surface and extend thin, branching processes resembling filopodia. ARHGAP6 associates with actin filaments through an N-terminal domain and mediates the redistribution of F-actin from focal adhesions into the growing processes. Mutation of a conserved arginine residue in the rhoGAP domain inhibits loss of stress fibers but has little effect on process outgrowth. These results suggest that ARHGAP6 has two independent functions as a GAP with specificity for rhoA and as a cytoskeletal protein that promotes actin polymerization or remodeling. The N-terminus of ARHGAP6, which remains intact in the mutant animals, may be essential for its in vivo activity. Therefore a second targeting vector was integrated into the first exon of *Arhgap6* to delete the entire coding region using cre-*lox*P recombination. Germline transmission the floxed *Arhgap6* allele is in progress.

Program Nr: 2761 from the 1999 ASHG Annual Meeting

Non-radioactive Dideoxy Fingerprinting for Mutation Analysis of BRCA1 and BRCA2. *S. Preisler-Adams, B. Dworniczak, J. Wansch, J. Horst.* Universitaet Muenster, Humangenetik, Muenster, NRW, Germany.

Mutations in the breast cancer susceptibility genes BRCA1 and BRCA2 account for a substantial proportion of familial breast and ovarian cancer syndromes. Both genes are comprised of more than 20 exons and contain a large internal exon, which has to be subdivided for mutation analysis. Due to the multitude of PCR formats there is a demand for an appropriate prescreening method before performing direct sequence analysis. Thus, dideoxy fingerprinting (DDF), a hybrid between dideoxy sequencing and single strand conformation analysis (SSCA), has been applied to test whether it meets the respective requirements. A ladder of bands is generated by performing only one of the four standard dideoxy sequencing reactions and resolving the products by electrophoresis on a nondenaturing polyacrylamide gel. Performing radioactive dideoxy fingerprinting according to the published procedures a high sensitivity could be obtained. In order to avoid the use of radioactivity we tested a nonradioactive approach using Cy5 labeled sequencing primers and an automated sequencer (A.L.F.express, Pharmacia). All reactions were performed following a standardized protocol using M13 primers and ddG as termination nucleotide. The required temperature control of the polyacrylamide gel was achieved by bypassing the heater and external cooling. Using the nonradioactive DDF we were able to identify all known common BRCA1 and BRCA2 polymorphisms in heterozygous state as well as in both homozygous forms. Furthermore all tested BRCA1 and BRCA2 mutations - including six point mutations - were also detectable with nonradioactive DDF.

Program Nr: 2762 from the 1999 ASHG Annual Meeting

Development of a Sensitive and Efficient Testing Strategy for Mutations in the APC Gene Associated with Familial Adenomatous Polyposis. *T.L Price-Troska, G. Chong, K. Snow.* Molecular Genetics Laboratory, Mayo Medical Center, Rochester, MN.

Familial Adenomatous Polyposis (FAP) is a dominantly inherited autosomal disorder characterized by multiple adenomatous polyps of the colon and a high probability of developing colorectal cancer an early age. The APC gene, which contains mutations associated with FAP, contains almost 9kb of coding sequence. Mutations in FAP are distributed throughout the gene, meaning that mutation detection can be very labor intensive. Our laboratory has developed an efficient and sensitive testing strategy for detecting mutations in the APC gene. The APC gene is divided into 5 segments. Segment 1 (exons 1-14) is analyzed by single-stranded conformation polymorphism (SSCP) and conformation sensitive gel electrophoresis (CSGE) methods using multiplexed PCR products, and by the protein truncation test (PTT). Segments 2 to 5 (which are overlapping regions of exon 15) are analyzed by the PTT using DNA as the starting material. Sequencing is used to characterize alterations detected by the above methods. In screening 35 unrelated patients that had a diagnosis of classical FAP, the overall mutation detection rate was 89%. Of interest, 55% of these mutations were in exons 1-14, which is significantly higher than previously reported detection rates for that segment. This difference is probably due to the inefficient detection of mutations in segment 1 by the PTT method. In our study of cases where RNA was available, testing of segment 1 using RT-PCR and PTT detected only 5 of 10 mutations detected by CSGE and/or SSCP, and all mutations detected by the PTT of segment 1 were detected by CSGE and/or SSCP. To maximize efficiency of testing in the clinical laboratory, we divide testing into two tiers. The first tier includes PTT analysis for segments 2 and 3, and CSGE analysis for segment 1. If no alterations are observed after the first tier, testing is continued. The second tier includes SSCP and PTT for segment 1, 4 and 5. To date, we have identified 38 different nonsense or frameshift mutations, and 26 of these have not been previously reported.

Program Nr: 2763 from the 1999 ASHG Annual Meeting

Molecular Analysis of the Duchenne Muscular Dystrophy Gene in Colombian Individuals. *J.C. Prieto[1,5], G. Keyeux[1], F. Leturcq[2], C. Duran[1], C. Rodas[1], S. Bautista[1], D. Recan[2], L.M. Camacho[3], E. Espinosa[4], J.E. Bernal[1], M.L. Tamayo[1], M. Cediel[4], P. Garavito[1], J.C. Kaplan[2]*. 1) Inst de Genetica Humana, Univ Javeriana, Bogota, Cundinamarca, Colombia; 2) Inst Cochin de Genetique Moleculaire, Paris; 3) Clinica Marly,Bogota; 4) Inst Roosevelt,Bogota; 5) Hospital la Victoria.

Duchenne muscular dystrophy (DMD) is the most common and severe neuromuscular disease in man and has an X-linked recessive mode of inheritance. Approximately 60% of DMD/BMD mutations are major deletions and 10% represent duplications. The deletions are nonrandomly distributed, one extending over the first 20 exons and the second near the middle of the gene around exons 45 to 53. In this pilot study in Colombia, a total of 38 patients were diagnosed using standard clinical diagnostic criteria. Deletion testing was performed by multiplex PCR, using 17 primer sets. STR loci (5`DYS II, 3`DYS MSA, 44, 45, 49 and 50) were analised determined in 50 X-chromosome haplotypes from unrelated individuals and the frequencies of observed alleles, % Heterozygosity and PIC were determined. Five families with DMD or BMD patientes were referred by carrier detection for segregation analysis of highly polymorphic STR loci. Of the 38 patients,14 (38%) had deletions located in the two mutational hot spots, 11 of them (79% of deletions) in the distal hot-spot, and 3(21%) in the proximal hot spot. The frequency of deletions found in this study is lower than described in the Caucasian population and similar to Asiatic and Spanish populations. Preliminary results suggest a high proportion of small mutations in our country or other dystrophin-complex diseases. Five of the families studied were informative because the carrier mothers were heterozygous for some the markers. In one family, the affected boy suffered a detectable de novo deletion of the intron 49 marker. This boy is affected by a deletion including adjacent exons. The frequencies of all observed alleles, % Heterozygosity and PIC were estimated and compared in several ethnic groups. Results derived from this study are useful for carrier detection and genetic counseling in DMD/BMD.

Program Nr: 2764 from the 1999 ASHG Annual Meeting

Identification of a carboxy-terminal nuclear localization signal in dyskerin. *M. Qatanani, V. Gharibyan, H. Youssoufian.* Dept. Molecular and Human Genetics, Baylor College of Medicine , Houston, TX.

The X-linked form of the bone marrow failure syndrome Dyskeratosis congenita is caused by mutations in dyskerin, a 514 amino acid protein that is presumed to play a role in ribosome biogenesis. There are at least two potential nuclear localization signals in dyskerin. Here we report that dyskerin tagged with the human immunoglobulin IgG1 epitope localizes to the nuclei of transfected HeLa and COS-1 cells. Placement of a carboxy-terminal domain consisting of amino acids 467-475 and encoding KKEKKKSKK at the carboxy-terminus of FANCC, a 558 amino acid cytoplasmic protein deficient in Fanconi anemia complementation group C, showed that this motif is both necessary and sufficient to mediate nuclear entry. This is the first report of the subcellular localization of dyskerin. The location of a nuclear localization signal at the carboxy terminus suggests that otherwise stable or active truncated mutants will remain inactive by failing to reach the nucleus, a mechanism similar to mutations in the Werner syndrome helicase.

Program Nr: 2765 from the 1999 ASHG Annual Meeting

Increased glycogen load accelerates the course of the disease in the acid alpha-glucosidase knockout mice. *N. Raben, K. Nagaraju, E. Lee, P. Plotz.* Arthritis and Rheumatism Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD.

We have previously reported generation of a knockout mouse model of Glycogen Storage Disease Type II (GSDII), a recessively inherited deficiency of acid alpha-glucosidase (GAA), which in its most severe form results in abnormal intra-lysosomal accumulation of glycogen in multiple organs, cardiomyopathy and death in infancy (Pompe disease). The animals lacking GAA activity (GAA-/-) developed generalized glycogen storage and showed obvious clinical signs of muscle weakness by 8-9 months of age. The knockouts have been followed now for up to 20 months. In this time the mice developed a severe progressive muscle-wasting disorder remarkably similar to that in human infantile GSDII: massive glycogen accumulation in skeletal muscle, diaphragm, heart, and brain, cardiomyopathy, hypotonia, severe motor disability, and profound muscle wasting with striking spine hunching. The relatively slow progression of clinical signs in mice suggests that the process of structural muscle damage resulting from the accumulation of lysosomal glycogen is absolute, and not relative to the life span. In order to develop a model with an earlier onset of the disease, the knockouts were bred to transgenic mice that overexpress the human GlutI glucose transporter in skeletal muscle (Marshall et al, JBC, 1993). The elevated glucose transporter activity in transgenic mice was associated with a significant increase in glycogen concentration in skeletal muscle, but phenotypically the mice remained normal. The transgenic mice on a GAA knockout background (GlutI/GAA-/-) developed the first obvious clinical signs of muscle weakness and wasting significantly earlier than the knockouts - by 4-5 months of age. By 9-12 months of age, however, the strains become clinically indistinguishable. In contrast to the knockouts, in which the females were more affected then males, the time course and the severity of the disease in GlutI/GAA-/- were similar in females and males.

Program Nr: 2766 from the 1999 ASHG Annual Meeting

The R3531C mutation in the APOB gene does not segregate with hypercholesterolemia in a large family. *J-P. Rabès[1,2], M. Varret[1], M. Devillers[1], L. Villéger[1], P. Aegerter[3], M. Krempf[4], C. Junien[1,2], C. Boileau[1]*. 1) INSERM U383, Paris; 2) Service de Biochimie et de Génétique Moléculaire, Hôpital A. Paré, Boulogne; 3) Service de Biostatistique, Hôpital A. Paré, Boulogne; 4) Service de Nutrition, Hôpital Hôtel-Dieu, Nantes (FRANCE).

Familial Hypercholesterolemia (FH) and Familial ligand-Defective apolipoprotein B-100 (FDB) are dominantly inherited disorders leading to impaired low density lipoprotein (LDL) receptor/apolipoprotein B-100 (apo B-100) interaction, plasma LDL elevation and hypercholesterolemia (HC). Numerous mutations in the LDL receptor gene (LDLR) lead to FH. Conversely, only 3 mutations in the apo B gene (APOB) have been associated with FDB, the R3531C substitution being one of them. We previously identified the first French APOB-R3531C proband, a 40 yo woman with very high cholesterol, in a group of type IIa dyslipoproteinemic families. We report herein the finding of the same mutation with an identical APOB haplotype in her unaffected husband. Further investigation of the family at large revealed 8 other carriers of the mutation but also, the absence of cosegregation with HC. Six of the 10 subjects heterozygous for the R3531C mutation had total plasma cholesterol lower than the 97.5th percentile for their age and sex, and plasma cholesterol was not significantly higher in carriers compared to family members lacking the mutation, questioning the deleterious nature of this substitution. Two family members with similar high cholesterol were not carriers of the R3531C mutation, suggesting the implication of another major gene. Segregation analysis of polymorphic markers of the LDLR gene revealed statistically significant genetic linkage with HC and analysis of proband LDLR gene lead to the identification of a 664 proline to leucine defective mutation and its detection in all 6 hypercholesterolemic members of the family. Therefore, our results show that the family presents with FH and give evidence that the R3531C substitution in the APOB gene is not an allelic variant leading to FDB. Furthermore, a two-way analysis of variance shows no significant interaction between APOB-R3531C genotype and LDLR-P664L mutation on cholesterol.

Program Nr: 2767 from the 1999 ASHG Annual Meeting

Mutational screening of the cationic trypsinogen gene in a large cohort of patients with sporadic chronic pancreatitis reveals novel DNA variants. *O. Raguenes[1], P. Deprez[2], Ch. Verellen[3], A. Andriulli[4], I. Quere[1], J.M. Chen[1], C. Ferec[1]*. 1) Centre de Biogenetique, Universite, Hopital, ETSBO, Brest, France; 2) Service de Gastroenterologie, Clinique Universitaire Saint Luc, Belgium; 3) Centre de Genetique Humaine, Unite de Genetique Medicale, Universite Catholique de Louvain, Bruxelles, Belgium; 4) Laboratorio Di Ricerca, Istituto Di Ricovero E Cura A Carattere Scientifico, San Giovanni Rotondo, Italy.

Two missense mutations of the cationic trypsinogen gene have been associated with hereditary pancreatitis (HP). To explore whether the cationic trypsinogen could play a role in the sporadic disease, we performed mutational analysis of the gene in 312 patients with sporadic chronic pancreatitis using denaturing gradient gel electrophoresis and detected novel DNA variants, all of which were absent in 400 control chromosomes. 1) In contrast to the single G>A transition resulting in an R122H (R117H in chymotrypsin numbering system) mutation identified in HP, a **GC**>**AT** 2-bp substitutions resulting also in an R122H was detected in 1 patient. This novel variant seems to arise as a gene conversion event: presences of **AT** at the corresponding positions of two other trypsinogen genes and a *Chi*-like sequence in the 3' proximity of the mutation. This finding also raises practical concerns: an R122H mutation, if arose as a gene conversion event, will be missed by the widely used PCR-*Afl* III digestion method (single G>A transition creates a novel restriction enzyme recognition site for *Afl* III—A¯CRYGT) and thus cause misleading interpretation. 2)A C>T transition resulting in an A16V detected in 2 patients. Since residue 16 is the signal peptide cleavage site, A16V may have an effect on the intracellular transport of the mutated protein. 3) Other single nucleotide substitutions detected once in different individuals: except for G>A (E79K), C>G (P36R), G>A (G83E) and G>T (K92N) all resulted in a nonconservative amino acid substitution at a well conservated residue. Furthermore, that no frameshift, nonsense and splicing mutations were detected is consistent with a "gain-of-function" dominant disease. Our results indicated that genetic testing is warranted in sporadic pancreatitis.

Program Nr: 2768 from the 1999 ASHG Annual Meeting

Developmentally regulated expression of AIRE during human leukocyte differentiation. *C. Ramsey[1], J. Kaukonen[2], A. Palotie[3], L. Peltonen[1,4].* 1) Department of Human Genetics, UCLA School of Medicine, Los Angeles, CA; 2) Department of Clinical Chemistry, University of Helsinki, Finland; 3) Department of Pathology, UCLA School of Medicine, Los Angleles, CA; 4) Department of Human Molecular Genetics, National Public Health Institute, Helsinki, Finland.

Autoimmune PolyEndocrinopathy-Candidiasis-Ectodermal Dystrophy (APECED) is a rare autosomal recessive disease enriched in Finnish, Iranian Jewish, and Sardinian populations. The APECED gene was previously identified and assigned to chromosome 21q22.3 by positional cloning. The causative gene, AIRE, is 2.25 kb long and northern blots show expression in thymus, pancreas and adrenal cortex. The predicted primary protein sequence of AIRE contains a proline rich region, two PHD-type zinc finger motifs, a nuclear localization signal and a newly identified SAND domain. To investigate the mechanism of autoimmunity with respect to AIRE, we monitored its expression in both peripheral blood cells and thymocytes. We examined the expression of AIRE mRNA in enriched populations of CD34+ peripheral leukocytes, B-cells, T-cells, monocytes, granulocytes, and thymocytes by RT-PCR. The AIRE mRNA is detected in peripheral CD34+ cells and T-cells. To characterize the expression of AIRE during T-cell maturation, we examined established human T-cell lines which were arrested at distinct stages of thymocyte maturation (in the order ARR-> MOLT-> CEM->Hut78), as well as one mature T-cell line, Jurkat, and compared the expression of AIRE in these cell lines with normal peripheral T-cells. Positive RT-PCR product was detected only with Hut78 cells; no product was observed from the other T-cell lines. These data are consistent with the previously reported expression of AIRE in mature medullary thymocytes, but not in immature cortical thymocytes. Interestingly, mature Jurkat cells did not express detectable level of AIRE mRNA, unlike normal peripheral T-cells. Thus, our data suggests that the AIRE mRNA is expressed in early hematopoietic development in pluripotent CD34+ cell leukocytes; AIRE expression is down regulated during T-cell maturation in the thymus, and then increases again in mature thymocytes and peripheral T-cells.

Program Nr: 2769 from the 1999 ASHG Annual Meeting

Members of a novel gene family on Xp22.3 are deleted in patients with X-linked non-specific mental retardation.

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Genotype-phenotype correlations in male patients with a partial nullisomy of the X chromosome have suggested the existence of at least one locus involved in X-linked non-specific mental retardation (MRX) on Xp22.3. Previous deletion mapping has defined that the critical region for this gene resides between markers DXS1060 and DXS1139, a region encompassing roughly 1.5 Mbp of DNA. Analysing the DNA of sixteen males with Xp deletion, we have narrowed down this MRX critical region to an interval of about 15 kb. Through exon amplification and the sequencing of two different cosmids which cover this region, a gene, VCX-A (Variably charged, X chromosome mRNA on CRI-S232A), was isolated. VCX-A consists of three exons. Due to a variable number of tandem repeats in exon III, the size of the predicted protein varies between 186 to 226 amino acids. VCX-A belongs to a gene family which contains at least four paralogs on Xp22.3 (VCX-A, B, B1 and C), and one on the Yq11.2 (VCY). Known motifs or domains were not detected in the gene sequence, and the function of VCX remains unknown at the present time. We found that all individuals with normal intelligence either have VCX-A or VCX-B or both, while all cases with MR lack both VCX-A and B, suggesting that one intact copy of either VCX-A or VCX-B is sufficient to maintain normal mental development.

Program Nr: 2770 from the 1999 ASHG Annual Meeting

Duplication of Chr. 17p13 in a patient affected with peripheral neuropathy, mental retardation and cranial dysmorphy. *B.W. Rautenstrauss[1], L. Brecevicz[1], D. Schweitzer[1], W. Voss[2], E. Gebhart[1]*. 1) Institute of Human Genetics, FAU Erlangen-Nuremberg, Erlangen, Bavaria, Germany; 2) Sozialpaediatrisches Zentrum, Janusz-Krozak-Allee 8, 30173 Hannover.

Charcot-Marie-Tooth disease type 1 A (CMT1A) is the most frequent form of autosomal dominantly inherited peripheral neuropathies. Typical clinical signs are reduced nerve conduction velocity, pes cavus deformity, gait disturbances and on sural nerve biopsy signs of de- and remyelination. Rarely patients show additional features like mental retardation. Most frequently a 1.5-Mb duplication in Chr. 17p11.2 including the PMP-22 gene is observed as cause of CMT1A. For some cases with additional mental retardation a partial or full duplication of the Smith-Magenis region was observed. The patient reported here has typical symptoms of a CMT disease like reduced NCV, on sural nerve biopsy signs of de- and remyelination and a pes cavus deformation. Additionally this patient is of short stature, has a cranial dysmorphy and is mentally retarded, at age of 7 years he did never learn to speak. Peripheral blood was used for the inital test on a CMT1A duplication in order to clarify the peripheral neuropathy, but this could be excluded by means of EcoRI/SacI Southern hybridisation (probe pLR7.8) and two color interphase fluorescence in situ hybridisation (FISH) with probes c132G8 (PMP-22) and cRCNeu. Point mutation screening in PMP-22, MPZ, Cx32 and EGR2 is ongoing. Duplication or deletion of the Smith Magenis region was excluded by two color FISH (fli-probe) as well as for the Miller-Dieker region. Finally by comparative genomic hybridisation (CGH) an extended duplication in chr. 17p13 was identified. This type of duplication in combination with a similar complex phenotype was described only once up to now in the Human Cytogenetics Database indicating the possibility of a contigous gene syndrome.

Program Nr: 2771 from the 1999 ASHG Annual Meeting

Mutation identified in crystallin gene in a family with zonular pulverulent cataract. *Z. Ren[1], A. Li[1], B.S. Shastry[2], T. Padma[3], R. Ayyagari[4], M.H. Scott[1], M.I. Kaiser-Kupfer[1], J.F. Hejtmancik[1].* 1) National Eye Institute, National Institutes of Health, Bethesda, MD; 2) Eye Research Institute, Oakland University, Rochester, MI 48309-4480; 3) Department of Genetics, Osmania University, Hyderabad,India; 4) Department of Ophthalmology, University of Michigan, Ann Arbor, MI.

Autosomal dominant zonular pulverulent cataracts were described in 48 members of an American family of European extraction over seven generations by Scott et al., Ophthalmology, 1994. We have mapped this locus to a 19 cM interval including the gamma-crystallin gene cluster on chromosome 2q33-q35. Sequencing and allele-specific oligonucliotide analysis of the pseudo gamma E-crystallin promoter region from individuals in the pedigree suggested that activation of the pseudo gamma E-crystallin gene with synthesis of an unstable crystallin is unlikely to be the cause of the cataract formation. In order to further investigate the cause of these cataracts and the underlying genetic mechanism, other gamma-crystallin genes were studied. Genomic DNA samples from affected family members and controls were analyzed by PCR with exon specific primers for the gamma C- and gamma D-crystallin genes, the most highly expressed human gamma-crystallins, and subsequently sequenced. The gamma D-crystallin gene showed no abnormalities, but a five base pair duplication within exon 2 of gamma-C crystallin was found in affected individuals and absent from unaffected individuals. The mutation disrupts the reading frame of the gamma C-crystallin coding sequence and is predicted to result in synthesis of an unstable gamma C-crystallin with the first Greek key motif followed by 52 random amino acids. This finding suggests that association of mutant beta gamma-crystallins into oligomers is not necessary to cause cataracts and may give us new insights into the genetic mechanism of cataract formation.

Program Nr: 2772 from the 1999 ASHG Annual Meeting

Novel mutations in a-**mannosidosis.** *H.M.F. Riise[1], T. Berg[2], H. Klenow[3], G. Evjen[4], E. Breines[3], O.K. Tollersrud[4], O. Nilssen[3]*. 1) Department of Human Genetics, UCLA, Los Angeles, CA, USA; 2) Department of Chemical Pathology, Womens and Childrens Hospital, Adelaide, Australia; 3) Department of Medical Genetics, University Hospital of Tromsø, Tromsø, Norway; 4) Department of Medical Biochemistry, University of Tromsø, Tromsø, Norway.

Lysosomal a-mannosidase (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 and cat. 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. Recently, we reported 23 disease causing mutations and six single nucleotide polymorphisms in the LAMAN gene of 42 unrelated a-mannosidosis patients mainly of European origin. In this work, 24 additional patients were analyzed by screening for known mutations as well as by automated DNA sequencing of the 24 LAMAN exons and exon-intron borders. Sixteen novel mutations; five missense, three nonsense, three splice site and five small insertions/deletions, were identified. In total, the 39 mutations were detected on 103 of the 132 alleles (78%). Most of the mutations were private or occurred in two or three families, except for one missense mutation, R750W, that was detected on 31 alleles (23.5%) in patients from 11 different countries. Haplotype analysis using four intragenic SNPs revealed that the 750W allele existed on three different haplotype backgrounds. The majority of the alleles (17 out of 20) shared the same haplotype, indicating that the 750W mutation probably spread by founder effects. The two other associated haplotypes could have emerged from the ancestral haplotype by single recombination events or, alternatively, resulted from recurrent mutations.

Program Nr: 2773 from the 1999 ASHG Annual Meeting

Darier Disease - Novel Mutations and a silent Polymorphism in ATP2A2. *F. Ringpfeil[1], A. Raus[1], J.J. Uitto[1], J.J. DiGiovanna[2,3], S.J. Bale[2], G. Richard[1]*. 1) Dept Derm, Jefferson Univ, Philadelphia, PA; 2) Gen Stud Section, NIAMS, NIH, Bethesda, MD; 3) Dept Derm, Brown Univ, Providence, RI.

Darier disease (DD) is a progressive, autosomal dominant genodermatosis with variable, but often debilitating skin manifestations. It may be associated with a range of neurological problems. Acantholysis of the epidermis is a histological hallmark suggesting impaired functionality of desmosomes. Recently, Sakuntabhai et al. (Nat Genet 21: 271-7, 1999) identified mutations in ATP2A2 in individuals with DD. ATP2A2 is a slow-twitched calcium pump that is widely expressed and controls cytosolic calcium levels. Thus it may confer a direct effect on the established calcium-dependant assembly of desmosomes. We screened the coding sequence of ATP2A2 in over 20 European and American families using conformation sensitive gel electrophoresis, and direct automated sequencing. To date, we identified 11 distinct mutations. We detected 7 missense mutations, 1 nonsense mutation, and small 3 in-frame deletions, all of which are novel with the exception of K683E. In addition, a silent polymorphism (nt.2172 G to A) with an allele frequency of 13.2% was found in exon 15. In accordance with previous reports, most pathogenic mutations are private. Our mutations are dispersed over different exons and are predicted to affect the upstream stalk, the transduction, phosphorylation, and ATP-binding sites, as well as the transmembrane and hinge domains. Two different missense mutations affect codon K683 changing this residue to arginine or glutamic acid in the corresponding hinge domain. Interestingly, K683E has been reported twice so far (Ruiz-Perez et al., abstract, JID in press), implying that this residue is critical for the function of the protein. To establish genotype-phenotype correlation, we compared molecular data with phenotypic features, such as severity and type of disease, occurrence of mucosal involvement, superinfections, or association with mental disorders. In our cohort, mutations in families with severe disease afflict the cytoplasmic transduction and phosphorylation domains, and the transmembrane regions, respectively. However, data are still too limited to draw further conclusions.

Program Nr: 2774 from the 1999 ASHG Annual Meeting

Protein expression of *COCH*, the DFNA9 sensorineural deafness gene, in human fetal cochlea and vestibule. *N.G. Robertson[1,2], J.S. Lin[1,2], C. Lee[1,2,3], H.L. Rehm[1,2,3], A. Shahsafaei[1], J.C. Aster[1,3], C.C. Morton[1,2,3]*. 1) Dept Pathology; 2) Dept Ob/Gyn, Brigham & Women's Hosp; 3) Harvard Medical School, Boston, MA.

The human *COCH* gene was shown previously to have four different missense mutations in American and European families with DFNA9, an autosomal dominant sensorineural hearing loss with vestibular dysfunction. To understand the functional role of *COCH*, we have developed a polyclonal antibody against the human Coch protein. The Coch peptide used as the immunogen consists of the Coch N-terminal factor C-like domain (Coch-FC peptide), which is mutated in DFNA9 patients. The von Willebrand type A-like domains present in *COCH* were omitted from the peptide to avoid possible cross-reactivity with other secreted proteins containing vWFA domains, such as collagens expressed in the cochlea.

The Coch-FC peptide expressed with an N-terminal histidine tag was purified over a Ni2+ column and used to immunize chickens. Antibodies isolated from immune eggs recognized the Coch-FC peptide and *in vitro* translated full-length Coch protein (~60 kD) on Western blots, whereas pre-immune serum did not react.

Using the a-Coch antibody, we performed immunohistochemistry on paraffin sections from 18-week-old human fetal cochlear and vestibular organs. High levels of staining were detected in the extracellular matrix of the spiral limbus, the area adjacent to collagen fibrils of the spiral ligament, the osseous spiral lamina along the fibers of the cochlear nerve, the spiral ganglion, and the epithelium and stroma of the saccular macula. This localization parallels the previously shown pattern of *Coch* mRNA expression in the chicken inner ear and matches the sites with characteristic acidophilic deposits observed in the cochlear and vestibular sections of individuals with DFNA9. The a-Coch antibody is a valuable biological tool to assess whether the mutant Coch protein is contained within the acidophilic deposits seen in DFNA9 and to investigate further the mechanism underlying this histopathology.

Program Nr: 2775 from the 1999 ASHG Annual Meeting

ARE A4336G POINT MUTATIONS AND mtDNA REARRANGEMENTS INVOLVED IN ALZHEIMERS DISEASE AETIOLOGY? *B. Rodriguez-Santiago[1], M. Gomez Zaera[1], J. Casademont[2], V. Nunes[1]*. 1) Med. and Mol. Genetics Center-IRO, Avia.Castelldefels km 2'7,08907 Hosp. Llobregat, Barcelona, Spain; 2) Muscular Research Unit, Department of Internal Medicine, Hospital Clnic, University of Barcelona, Villaroel 170, 08036 Barcelona, Spain.

Alzheimers disease (AD) is associated with defects in mitochondrial function. AD has a complex aetiology, and it is widely considered that genetic factors, acting independently or in concert with other genetic and/or environmental factors, modify the risk of developing the disease. There is controversy about the role of the mitochondrial tRNA (Gln) sequence variant (A4336G transition). This mutation was reported to occur with increased frequency in individuals with AD (Egensperger et al, Hutchin and Cortopassi, i.e.) but other groups reported no association (Wragg et al) or it being a polymorphism variant (Leroy et al). We have examined the mtDNA A4336G transition in necropsy samples of cerebellum, hipocampus and cortex from 7 confirmed cases of AD and from 9 age-matched controls without clinical or histological evidence of neurodegenerative disease. The mtDNA (A4336G) mutation was present in all necropsy regions from 2 AD cases and in another AD case the three regions analyzed showed an abnormal digestion pattern. All control samples showed a normal pattern. This mutation was also analyzed in blood samples from 6 AD patients and 8 age-matched controls: neither the AD nor the control samples harbored the mutation. We have also examined the presence of large mtDNA rearrangements in the brain necropsy samples by long PCR and southern-blot analysis. There was no difference between AD and controls. Our data supports the involvement of the A4336G mutation in Alzheimers disease.

Program Nr: 2776 from the 1999 ASHG Annual Meeting

Deletion of a novel Zinc finger gene in Smith-Magenis Syndrome. *F. Romero-Pastrana[1], A.M. Brown[1,2], C.E. Schwartz[1], A.K. Srivastava[1]*. 1) Center for Molecular Studies, J. C. Self Research Institute of Human Genetics, Greenwood Genetic Center, Greenwood, SC; 2) Present address: Div. of Endocrinology, Med. Univ. of South Carolina, Charleston, SC.

Smith-Magenis syndrome (SMS) is a clinically distinctive mental retardation syndrome associated with a complex and heterogeneous phenotype. The features include developmental and growth delay, brachycephaly, flat midface, short broad hands, syndactyly, and characteristic behavioral problems. Ear abnormalities, sleep disorder, conductive hearing loss, and moderate to sever myopia are also seen among SMS patients. The complex phenotype is associated with variable hemizygous deletions and duplications in chromosomal band 17p11.2. A repeated gene cluster, SMS-REP, has been identified, which flanks the genomic region commonly deleted in SMS patients. (Chen et al., 17, 154-163, 1997).

We have isolated most of the SMS critical region (D17S58-D17S445) in YAC clones and have analyzed a series of SMS patients to facilitate the cloning of the gene(s) involved in SMS. In a search for local transcripts, we have identified three novel zinc finger genes (ZNFa, ZNFb and ZNFc) from the SMS region. The transcripts corresponding to two of these zinc finger genes, ZNFa and ZNFb, have been assembled and characterized. Both genes consist of multiple exons and encode novel ZNF proteins containing 10-14 C2H2-type finger motifs. We have verified the deletion of at least one zinc finger gene, ZNFa, in several SMS patients. ZNFa is found to be expressed differentially in human fetal and adult tissues, with relative abundance much in fetal and adult brain. Deletion of one or all the ZNFs in this cluster can now be tested as contributory to the multiple anomalies observed in SMS patients.

Program Nr: 2777 from the 1999 ASHG Annual Meeting

Novel missense mutations in the DKC1 gene in patients with dyskeratosis congenita. *K. Rostamiani[1], N. Heiss[2], A. Poustka[2], A.B. Metzenberg[1]*. 1) Biology, Cal State Northridge, Northridge, CA; 2) Dept. of Molecular Genome Analysis, Deutsches Krebsforschungszentrum, Heidelberg, Germany.

Dyskeratosis congenita (DKC) is a rare, lethal inherited syndrome characterized by abnormal skin pigmentation, nail dystrophy, and mucosal leukoplakia. Progressive pancytopenia and bone marrow failure are the most common causes of death, and patients also have an increased risk for infections and have an increased incidence of malignancy. The X-linked form of the disease (MIM 305000) has been shown to be caused mainly by mutations in the DKC1 gene, which lies 40 kb centromeric to the MPP1 gene in Xq28. The product of DKC1 is predicted to be a 514-amino-acid protein, called dyskerin. Dyskerin is highly conserved through evolution, from bacteria to humans, and is believed to be a nucleolar protein which functions in rRNA biogenesis.

DNA was extracted from peripheral blood from each of nine unrelated male patients with DKC. Each of the 15 exons and flanking intron/exon boundaries of the DKC1 gene were amplified by PCR, and sequenced revealing one previously observed mutation, as well as unique missense mutations in three patients, including one at a potential mutational hotspot. Two of the mutations, ntC206T (T49M) in Exon 3 and the new ntG1025A (R322Q) in Exon 10, are C to T transitions in CG dinucleotides, which are common hotspots for such mutations. The T49M mutation has been previously reported, as have recurring CG->TG mutations in two other exons of the DKC1 gene. The other two novel missense mutations are ntC1009T (L317K) in Exon 10, and ntC1210T (P384S) in Exon 11. Five of the patients had wild-type DKC1 sequences, including one with an affected brother. In the absence of informative linkage analysis in the families of these affected males, it is not possible to predict whether it is likely that they have mutations in the promoter regions, introns or 3'-UTRs of the DKC1 gene, or whether they have mutations in another X-linked or even an autosomal locus. Further studies are essential for improving the usefulness of molecular testing in DKC for clinicians and families.

Program Nr: 2778 from the 1999 ASHG Annual Meeting

Human EST screening provides 96 new nuclear candidate genes for mitochondrial disorders. *A. Rotig, C. Mugnier, P. Rustin, A. Munnich.* INSERM U393, Hopital Necker, Paris, France.

Mitochondrial disorders represent an increasing group of metabolic diseases. Mutations in mitochondrial as well as nuclear genes encoding respiratory chain proteins have been recently described. Interestingly, mutations in genes encoding proteins involved in respiratory chain assembly, mitochondrial proteins traffic or mitochondrial iron metabolism have been shown to lead to mitochondrial disorders as well. Yet, only few genes encoding proteins involved in respiratory chain assembly, protein trafficking, and mtDNA maintenance have been identified in human. However, several hundred of those nuclear genes have been described in yeast. These yeast nuclear genes constitute an invaluable tool for characterization of human genes possibly responsible of mitochondrial disorders. In order to identity the human counterparts of these genes, we developed a systematic "cyber-screening" approach combining different yeast protein databases and the Genbank dbEST database. Using 312 yeast protein sequences as templates, we screened the human dbEST using the BLAST similarity searching program and identified more than 140 groups of human EST likely to represent orthologs of yeast genes with a significant P value ($p<10^{-5}$). This collection of human genes possibly involved in mitochondrial turnover will help identifying nuclear genes responsible of mitochondrial disorders.

Program Nr: 2779 from the 1999 ASHG Annual Meeting

Characterization of the murine homolog of the Treacher Collins Syndrome gene product, treacle. *S. Rujirabanjerd[1], S.T. Winokur[2], R. Shiang[1]*. 1) Virginia Commonwealth University, Richmond, VA; 2) University of California, Irvine, CA.

Treacher Collins Syndrome (TCOF1) is an autosomal dominant disorder of craniofacial development. Although the gene has been cloned, the functional and biochemical properties of the protein, treacle, are still unknown. Treacle is thought to be a member of a class of nucleolar phosphoproteins from its predicted protein sequence. The murine homologue of the TCOF1 gene has also been identified. The murine treacle is 1320 amino acids in length with a predicted molecular weight of 133 kDa. It also contains several potential sites for CKII (casein kinaseII) phosphorylation. Both the human and mouse proteins have been previously shown to localize to the nucleolus. To determine whether murine treacle is phosphorylated, we performed *in vitro* dephosphorylation experiments with non-specific calf intestine alkaline phosphatase (CIAP) using cell lysates from mouse P19 cells, an embryonic carcinoma cell line. The results were visualized using anti-treacle antibodies after electroblotting. The untreated protein was found to be 205 kDa while the protein treated with CIAP was 190 kDa. The smaller protein was not observed when phosphatase inhibitors were added. We concluded that the murine treacle is highly phosphorylated. The molecular weight of both forms of treacle is larger than its predicted molecular weight. This may be caused by aberrant migration of the protein on the gel or other post-translational modification of the protein. To further determine if the 190 kDa product is a true unmodified product of the murine treacle, *in vitro* transcription/translation was performed using a rabbit reticulocyte lysate system and the protein was labeled by ^{35}S-methionine. The result of this experiment confirms that the 190 kDa protein is the unphosphorylated and unmodified form of treacle. Since the predicted protein sequences contain several sites for CKII phosphorylation, experiments to determine whether CKII mediates phosphorylation were performed. The unphosphorylated form of treacle was incubated with CKII *in vitro*. The results show that treacle can be phosphorylated by CKII. This work confirms that treacle is a member of a nucleolar phosphoprotein family.

Program Nr: 2780 from the 1999 ASHG Annual Meeting

The PHEX locus-specific mutation database: A centralized location and search tool for mutations causing X-linked hypophosphatemia. *Y. Sabbagh[1], A.O. Jones[1], P. Nowacki[3], H.S. Tenenhouse[1,2]*. 1) Dept Biology; 2) Dept Pediatrics and Human Genetics, McGill University, Montreal Children's Hospital Research Institute, Montreal, PQ, Canada; 3) Centre for Genome Research, McGill University, Montreal General Hospital Research Institute, Montreal, PQ, Canada.

X-linked hypophosphatemia (XLH) (OMIM 307800) is a dominant disorder of phosphate homeostasis associated with growth retardation, rachitic and osteomalacic bone disease, hypophosphatemia, and renal defects in the reabsorption of filtered Pi and the metabolism of vitamin D. The gene responsible for XLH was identified by an International Consortium by positional cloning, and was designated *PHEX* (formerly *PEX*) to depict a Phosphate regulating gene with homology to Endopeptidases on the X chromosome. Approximately 86 mutations in the *PHEX* gene have been reported in XLH patients. However, with time more mutations will be identified, and a novel way to access this information is through a database search tool. PHEXdb (http://data.mch.mcgill.ca/phexdb) is a relational database on presently published mutations in the *PHEX* gene. This site is dedicated to the collection and distribution of information on all PHEX mutations in one location that is easily accessible to the scientific community. PHEXdb will allow online access where users will be able to search the database by mutation, which includes mutation name, mutation type, and codon name; by phenotype, which includes inheritance, X-linked or sporadic, clinical and biochemical phenotype; and by authors who published or submitted mutations. Also PHEXdb provides a submission form that will allow newly identified mutations in the *PHEX* gene to be added to the database, thereby centralizing the information in one location. The PHEXdb homepage also includes links to information pages, including publications relevant to PHEX, XLH and mouse homologues (Hyp and Gy).

Program Nr: 2781 from the 1999 ASHG Annual Meeting

Mutational analyses and comparison of clinical phenotypes of six families with autosomal recessive juvenile parkinsonism (AR-JP). *M. Saito[1,2], M. Maruyama[2], T. Ikeuchi[2], H. Kondo[3], T. Yuasa[4], A. Ishikawa[5], S. Tsuji[2].* 1) Department of Neurology, National West Niigata Central Hospital, Niigata 950-2085, Japan; 2) Department of Neurology, Brain Research Institute, Niigata University, Niigata 951-8585, Japan; 3) Department of Neurology, Niigata National Hospital, Kashiwazaki 945-8585, Japan; 4) Department of Neurology, National Center of Neurologyand Psychiatry, Kohnodai Hospital, Ichikawa 272-8516, Japan; 5) Department of Neurology, Nishi-Ojiya National Hospital, Ojiya 947-0041, Japan.

Purpose; Recently, the causative gene for AR-JP was mapped on chromosome 6q, and a novel gene 'parkin' was identified. This study is aimed to analyze the genotype-phenotype correlations of six families with AR-JP. Methods; Six unrelated Japanese families (Pedigrees 455, 549, 552, 554, 992 and 1535) with AR-JP were investigated in this study. Blood samples were taken with informed consent from the family members. Exon deletions were detected by PCR amplification of individual 12 exons of the parkin gene, and were confirmed by genomic Southern blot hybridization analysis using a 1.4kb parkin cDNA fragment. To accurately determine the deleted exons in two alleles, gene dosage analysis of individual exons of parkin gene were performed using ABI Prism 7700 Sequence Detection System. Point mutations were identified by direct nucleotide sequence analysis of the RT-PCR products using total RNA extracted from the lymphoblastoid cell lines. Results; Deletions of exons 2-4 (Ped 549), exon 4 (Ped 455 and Ped 1535) and exons 6-7 (Ped 992) were identified. Gene dosage analysis revealed that affected individuals of Ped 455 was compound heterozygotes for one allele missing exon 2 and the other allele missing exons 2-4. Nonsense and missense mutations were identified in Ped 552 and Ped 554, respectively. The clinical features and the severity were similar among the affected individuals in the six pedigrees irrespective of the genotypes. Although the age at onset varies considerably among the affected individuals in the six pedigrees, we could not detect any correlations between the age at onset and the genotypes, suggesting involvement of other genetic or environmental factors in the age at onset.

Program Nr: 2782 from the 1999 ASHG Annual Meeting

A novel point mutation of tyrosinase gene causes atypical oculocutaneus albinism in Syrian hamster. *A. Sakamoto[1, 2], M. Abe[2], T. Masaki[1]*. 1) Div Biotech, Dept Biosci, Natl Cardiovasc Ctr, Osaka, Japan; 2) PRESTO, JST, Saitama, Japan.

BIO14.6 Syrian hamster, a representative animal model for hereditary cardiomyopathy, accompanies oculocutaneus albinism (OCA) with unique profile. At birth, BIO14.6 hamster has red eye-appearance with no melanin deposit in retinal pigment epithelium (RPE) but, as it grows, its eye-appearance becomes dark brown with gradual pigment accumulation in RPE.

We failed to identify any exons responsible for OCA in the genomic interval deleted in BIO14.6 hamster, which spans about 30 kb and includes the two first exons of delta-sarcoglycan (Sakamoto et al. FEBS Lett. 447:124-8. 1999). Thus we searched for genetic mutation of tyrosinase, a key enzyme for melanin production. The open reading frame of normal Golden hamster cDNA for tyrosinase, isolated from the whole eye cDNA library, comprised 1,590 nucleotides and was assumed to encode a type I single transmembrane polypeptide consisting of 530 amino acid residues with calculated molecular mass of 60,436. The amino acid identities of tyrosinases between hamster and human or hamster and mouse were 85.7 % or 90.1 %, respectively. RNA blot analysis detected tyrosinase transcript in the eye of BIO14.6 hamster, which was equal in size and amount to that of Golden hamster. Cloning and sequencing of BIO14.6 hamster cDNA for tyrosinase revealed single nucleotide change of T785C, which caused amino acid change of Leu262Pro. Leucine at this corresponding position of tyrosinases is conserved among human, mouse and chick, and this mutation has never been reported in OCA of any species. We established several pedigrees between Golden and BIO14.6 hamsters, where only F2 with homozygotic allele of (C/C) manifested OCA phenotype. We generated Hela cell lines stably expressing tyrosinase of Golden or BIO14.6 hamsters. Various enzymatic characteristics of BIO14.6 hamster tyrosinase are now being determined.

These data not only suggest that Leu262Pro mutation of tyrosinase causes atypical OCA in Syrian hamster but also indicate that BIO14.6 hamster also serves as a useful animal model for studying the effect of gradual pigmentation in RPE on visual function.

Program Nr: 2783 from the 1999 ASHG Annual Meeting

A CRX mutation in a Finnish family with dominant cone-rod retinal dystrophy. *E.M. Sankila[1,3], T.H. Joensuu[1,2], R.H. Hamalainen[1,2], N. Raitanen[1,2], J. Ignatius[4], B. Gormand[1,2].* 1) Department of Medical Genetics, Univ Helsinki, Helsinki, Finland; 2) Department of Molecular Genetics, The Folkhalsan Institute of Genetics, Helsinki, Finland; 3) Department of Ophthalmology, Helsinki University Hospital, Helsinki, Finland; 4) Department of Neurophysiology, Jorvi Hospital, Espoo, Finland.

Cone-rod retinal dystrophy (CRD) is a progressive retinal degeneration clinically characterized by early loss of colour vision and visual acuity followed by nyctalopia and loss of peripheral vision. Autosomal dominant, recessive and X-linked inheritance have been described. Recently, an autosomal dominant CRD locus was assigned to chromosome 19q13 by linkage and subsequently, mutations in a photoreceptor-specific homeobox gene (CRX) were found in CRD patients. CRX has also been implicated in Leber's congenital amaurosis and retinitis pigmentosa. We have studied a Finnish CRD family with 21 affected family members in six generations. CRD in this family is characterized by a relatively early age of onset (2-6 years), early loss of blue-green colour vision and progressive visual failure. Typically, macular pigmentation, bull's eye lesion and later changes resembling retinitis pigmentosa are seen in ophthalmoscopy. The assignment of a CRD locus in 19q13 prompted our clinical follow-up studies and linkage analyses of the Finnish CRD family. Blood samples were collected from 14 affected and 10 unaffected consenting family members. Linkage analyses with chromosome 19 markers revealed positive lod scores with two markers flanking the CRX locus, namely D19S178 and D19S246 (theta 0.1, zmax 2.12 for both loci). The sequence analysis of the CRX gene in the patients revealed a G to A transition resulting in a Glu80Lys substitution. Previously, a Glu to Ala change at this first position of the recognition helix has been reported in a dominant CRD family.

Program Nr: 2784 from the 1999 ASHG Annual Meeting

Transmission of an *AZFc* deleted Y chromosome from a non-mosaic male to his three infertile sons. *N. Saut[1], P. Terriou[2], A. Navarro[1,3], C. Guillemain[4], N. Levy[1,3], M.J. Mitchell[1]*. 1) INSERM U.491, Faculte de medecine, Marseille, FRANCE; 2) Institut de Medecine de la Reproduction, Marseille, FRANCE; 3) Laboratoire de Genetique Moleculaire, Hopital de la Timone, Marseille, FRANCE; 4) Biologie de la Reproduction, Hopital de la Conception, Marseille, FRANCE.

Several studies have found that the *AZFc* interval of the human Y chromosome is deleted in between 3-20% of males with an idiopathic infertility associated with a severely reduced sperm count. These large deletions of the distal Yq euchromatin remove members of several testis-specific multi-copy gene families, such as *DAZ*, which therefore represent candidate azoospermia genes. We have analysed a cohort of 160 azoospermic or oligospermic patients (sperm concentration <20 x 10^6/ml) with a panel of 24 markers using the polymerase chain reaction (PCR). We identified 9 *AZFc* deletions in patients with <5 x 10^6 sperm/ml (9%; n=102). No deletions were detected in patients with 5-20 x 10^6 sperm/ml. Southern analysis revealed that all 9 *AZFc* deletions removed the entire *DAZ* gene cluster. FSH levels were elevated in all cases. Interestingly, three of the patients with *AZFc* deletions are brothers (2 oligospermic/azoospermic, 1 azoospermic), suggesting that the deleted Y chromosome was transmitted by their father. The father's DNA was tested exactly as for his sons, by PCR and Southern, and at this level of resolution he and his sons were determined to possess an identically deleted Y chromosome. No evidence of mosaicism was seen in the father by PCR with 13 *AZFc* markers on DNA from blood lymphocytes. The father was 26 years old at the birth of his first son and 29 years old at the birth of his third son. This, therefore, shows that efficient spermatogenesis can be sustained over a period of 3 years in a male with a Y chromosome bearing a large *AZFc* deletion which removes all copies of the candidate azoospermia gene *DAZ* (*Deleted in azoospermia*). Molecular characterisation of this exceptional family is continuing with the aim of defining the precise extent of the deleted interval in the father and his sons.

Program Nr: 2785 from the 1999 ASHG Annual Meeting

A stem-loop mutation in intron 18 of the RB1 gene leads to aberrant splicing. *H. Scheffer[1], P. van der Vlies[1], M. Burton[1], E. Verlind[1], E. Leter[2], C.H.C.M. Buys[1].* 1) Dept Medical Genetics, State Univ Groningen, Groningen, Netherlands; 2) Dept Clinical Genetics, Free Univ Hospital Amsterdam, Amsterdam, Netherlands.

In 74 out of 92 familial and/or bilateral retinoblastoma patients we have identified the causative germ line mutation in the RB1 gene. In 26% of the mutations identified the splicing of the RB1 primary transcript is disturbed by changing the splice acceptor site or the splice donor site sequences. In a sporadic patient a T to C transition at nucleotide position +11 from the exon 18 to intron 18 junction was identified. The unaffected parents were not available for analysis. This intronic mutation was not detected in any other DNA sample we studied. It restores a perfect inverted repeat sequence of the exon 18 splice donor site, and thus leads to stabilization of a stem-loop with this splice site at the primary transcript level. Its use as a splicing signal will at least partially be abolished. An altered ratio of normally versus aberrantly spliced transcripts has been detected in this patient.

Program Nr: 2786 from the 1999 ASHG Annual Meeting

Characterization of retinoic acid-sensitive developmental genes using an induction gene trap approach. *M. Schlicker[1], O. Batista[1], K. Recker[1], A. Voigt[1], W. Wurst[2], I. Hansmann[1]*. 1) Martin-Luther-Universitaet, Humangenetik und Med. Biologie, Halle/Saale, Germany; 2) GSF-Forschungszentrum fuer Umwelt und Gesundheit, Molekulare Neurologie, Neuherberg, Germany.

In man and mammals prenatal exposure to retinoic acid (RA) and/or retinoids (RX) results in characteristic congenital anomalies. 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 intracellular RA/RX binding proteins and 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 relevant for mouse and human development. By RACE-PCR of trapped ES clones we identified two novel genes that are expressed in early brain and heart. A 1 kb cDNA fragment of the 1st gene (EScD-1) identifies a brain-specific 15 kb transcript in new born mice. The 2nd gene (EScD-2), represented so far by a 400 bp cDNA-fragment is expressed in fetal brain (2.5 kb) and heart (1.4 kb). The expression of both transcripts has been identified prenatally from day 8 p.c. onwards at least to birth. The heart-specific transcript of 1.4 kb is expressed also in adult heart. Both cDNA sequences, EScD-1 and EScD-2 have been completely sequenced and and show single open reading frames. Searches for human homology revealed that parts of EScD-2 are represented within a human cosmid and cDNA both mapping to chromosome 16p13.3. Breakpoints within that chromosome segment are known to be associated with cardiac anomalies. Using EscD-2 cDNA as a probe we isolated a human BAC clone to verify the localization of the respective gene in 16p13.3. The corresponding FISH analysis and a detailed analysis of the expression patterns of EScD-1 and EScD-2 as well as the cloning of the respective human genes is in progress. These data as well as the study of the mouse gene trap phenotypes will give information about the involvement of the two genes in specific human disorders of brain and/or heart. (Supported by DAAD and DFG).

Program Nr: 2787 from the 1999 ASHG Annual Meeting

The cardiac pacemaker channel gene, HCN-2, is not linked with congenital sinus node dysfunction and AV-conduction block. *E. Schulze-Bahr[1,2], E. Morhofer[2], M. Borggrefe[1,2], P. Vogt[3], S. Kotthoff[3], H. Funke[2], G. Breithardt[1,2]*. 1) Dep. of Cardiology, Hospital of the Univ. Muenster, Muenster, Germany; 2) Institute for Arteriosclerosis Research at the Univ. Muenster; 3) Dep. of Pediatric Cardiology, Hospital of the Univ. Muenster.

Sinus node dysfunction is the major indication for pacemaker implantation. The underlying cause remains in 40-60% ("idiopathic") when there is no evidence for organic heart disease. Recently, the HCN-2 gene encoding the fast-activating component of the cardiac pacemaker current I(f)has been cloned and localized on chromosome 19p13.3. We identified one large (n=33 family members) and one small (n=4) German descendent with congenital sinus node dysfunction binodal disease and, thus performed linkage analysis with microsatellite markers at chr. 19p13.3. Using markers D19S216-D19S413-D19S604-D19S209, encompassing the HCN-2 locus, we did not find an haplotype co-segregating with the disease. We continued in studying further 15 candidate gene loci, primarily including ion channel gene loci in which the mutated genes are linked to distinct arrhythmias and hypothesized that mutations in these genes may cause allelic disorders including sinus node dysfunction. When investigating the LQT1-6 locus, the Kir locus, the NF-kappa as well as loci for dilatated cardiomyopathy we have not found linkage yet. Taken together, we have exluded the gene encoding the fast component of the pacemaker current from candidacy in congenital sinus node dysfunction. For further investigations, we now collect additional individuals or families with congenital sinus node dysfunction and/or AV-conduction block and perform genome-wide linkage analyses to identify the disease gene by combined linkage with SNP and microsatellite markers.

Program Nr: 2788 from the 1999 ASHG Annual Meeting

Functional analysis of the *PDS* gene product explains phenotypic variation in patients with Pendred syndrome and nonsyndromic hearing loss (DFNB4). *D.A. Scott[1,2], R. Wang[3], T.M. Kreman[3], J.M. McDonald[4], J.R. Marietta[4], R.J.H. Smith[4], L.P. Karniski[3,5], V.C. Sheffield[1,2].* 1) Howard Hughes Medical Institute; 2) Dept. Pediatrics; 3) Dept. Internal Medicine; 4) Dept. of Otolaryngology-Head and Neck Surgery, University of Iowa Hospitals and Clinics, and; 5) Iowa City Veterans Affairs, Iowa City, IA.

Mutations in the *PDS* gene are responsible for Pendred syndrome (early onset hearing loss and goiter), as well as autosomal recessive nonsyndromic hearing loss (ARNSHL) at the DFNB4 locus on chromosome 7q31. The *PDS* gene encodes a transmembrane protein, known as pendrin, which was hypothesized to transport sulfate. However, we have demonstrated, in both *Xenopus* oocytes and Sf9 cells, that pendrin does not transport sulfate but is a transporter of iodide and chloride. To determine if *PDS* mutations which result in Pendred syndrome are functionally distinct from those causing ARNSHL, we analyzed the transport properties of oocytes injected with modified *PDS* cRNA containing one of three common Pendred syndrome mutations, L236P, T416P, and E384G, and the I490L and G497S variants which are each homozygous in an inbred Indian family with ARNSHL. All of the Pendred syndrome mutations caused complete loss of pendrin iodide and chloride transport; oocytes injected with I490L cRNA showed near wild-type levels of ion transport (simple polymorphism); and oocytes injected with G497S cRNA showed residual activity compared to water injected controls and Pendred syndrome mutations. To identify additional *PDS* mutations associated with ARNSHL, we screened 20 individuals with apparent nonsyndromic hearing loss and dilated vestibular aqueducts (DVA), a common radiological finding in hearing loss. This screen revealed three individuals (15%) who carried one or more *PDS* mutations: V653A/+, L236P/V480D, T416P/+. Functional analysis of the V653A and V480D alleles revealed residual *PDS* activity. These data indicate that *PDS* mutations can be identified in a significant percentage individuals with nonsyndromic hearing loss and DVA and that residual pendrin function may postpone or eliminate the onset of goiter in *PDS*-related nonsyndromic hearing loss.

Program Nr: 2789 from the 1999 ASHG Annual Meeting

Structure of the SLC7A7 gene and mutational analysis of patients affected by lysinuric protein intolerance. *G. Sebastio[1], M.T. Bassi[2], M.P. Sperandeo[1], M. Riboni[2], G. Parenti[1], A. Buoninconti[1], M. Manzoni[2], B. Incerti[1], M. Di Rocco[3], P. Strisciuglio[3], I. Dianzani[3], R. Parini[3], A. Adami[3], C. Dionisi Vici[3], M. Candito[3], F. Endo[3], A. Pepe[1], A. Ballabio[2,4], G. Andria[1], G. Borsani[2]*. 1) Dept Pediatrics, Federico II Univ, Naples, Italy; 2) Telethon Institute of Genetics and Medicine, TIGEM-HSR, Milan, Italy; 3) LPI Collaborative Group; 4) Universita' Vita e Salute, San Raffaele, Milan, Italy.

Lysinuric protein intolerance (LPI; MIM 222700) is a rare autosomal recessive defect of cationic amino acid transport at the basolateral membrane of enterocytes and renal tubuli. We previously reported the isolation of SLC7A7, the gene mutated in LPI, and found mutations in four Italian pedigrees and Finnish patients (Nature Genet 21: 297-301, 1999). Here we report the genomic structure of the gene and the results of the mutational analysis in additional Italian, Tunisian and Japanese patients. The SLC7A7 gene consists of 10 exons; sequences of all the exon-intron boundaries are reported here. Twenty-five out of the 26 independent mutant alleles were characterized and 9 novel mutations were detected, including: 3 missense mutations, 242A to C (M1L), 390T to A (M50K) and 1399C to A (S386R); a nonsense mutation 967G to A (W242X); 2 splice mutations IVS3+1G to A and IVS6+1G to T; a single base insertion, 786insT; two 4-bp deletions, 455delCTCT and 1425delTTCT. Noteworthy, 242A to C causes the change of Met1 to Leu, a rare mutational event previously found in a few inherited conditions. We failed to establish a genotype/phenotype correlation. Actually, both intrafamilial and interfamilial phenotypic variability were observed in homozygotes for the same mutation. A strong mutational heterogeneity was observed in Italian patients (9 different mutations for 23 independent alleles), though they originate mainly from two restricted geographical areas. This recalls the so called 'Reunion paradox', that is the occurrence of different mutations for rare diseases in genetic isolates. The DNA based tests are now easily accessible for molecular diagnosis, genetic counseling and prenatal diagnosis of LPI.

Program Nr: 2790 from the 1999 ASHG Annual Meeting

Spectrum and frequency of PITX2/RIEG mutations in patients with Rieger syndrome and related ocular anomalies. *E.V. Semina[1], C. Funkhauser[1], P. Bitoun[2], W.L.M. Alward[3], S. Daack-Hirsh[1], B. Amendt[4], B. Ludwig[1], J.C. Murray[1,5]*. 1) Dept Pediatrics, Univ Iowa, Iowa City, IA; 2) Pediatrie Genetique, Hpital Jean Verdier, 93143 Bondy, FRANCE; 3) Dept Ophthalmology, Univ Iowa, Iowa City, IA; 4) Dept Phisyology, Univ Iowa, Iowa City, IA; 5) Dept Biol Sciences, Univ Iowa, Iowa City, IA.

Rieger syndrome (RIEG) is an autosomal dominant heterogeneous disorder with complete penetrance but variable expressivity, mainly characterized by craniofacial dysmorphism, malformations in the anterior chamber of the eye predisposing to glaucoma, dental and umbilical anomalies. We have previously identified a homeodomain- containing transcription factor gene PITX2 responsible for 4q25-linked cases of Rieger syndrome. The Pitx2 gene has also been shown to play a crucial role in determination of left/right patterning of visceral organs in vertebrates. Three different isoforms of PITX2 gene have been reported. The complete genomic sequence of the RIEG1/PITX2 gene was obtained and genomic structure of all of the isoforms was determined and used to design primers for PCR-based mutation search. We have now evaluated 30 individuals with RIEG and 98 with isolated anterior segment anomalies for mutations in PITX2 gene. Six additional mutations have been identified, four of which represent novel alterations of the gene. A total of 37% of Rieger syndrome patients and 1% of patients with isolated ocular anomalies were found to have PITX2 mutations in our sample. A composite of our and published findings shows that almost all mutations affect the homeobox region which encodes a homeodomain with a major role in target recognition and protein binding. Most of the alterations are unique with two repetitive mutations. We found no visible correlation between the location or type of mutation and clinical features of Rieger syndrome. Although there were no clear difference in the clinical picture between RIEG patients with PITX2 mutations and others, they tend to more frequently demonstrate gut malformations including omphalocele and Meckel's diverticulum. A search for mutations in additional patients with Rieger syndrome and related anomalies is in progress.

Program Nr: 2791 from the 1999 ASHG Annual Meeting

Molecular genetic analysis of 40 patients with glycogen storage disease type Ia: 100% mutation detection rate and 5 novel mutations. *H.H Seydewitz[1], D. Matern[2]*. 1) Children's Hospital, Albert-Ludwigs-University, Freiburg, Germany; 2) Division of Medical Genetics, Department of Pediatrics, Duke University Medical Center, Durham, NC.

BACKGROUND: The catalytic subunit of microsomal glucose-6-phosphatase (G-6-Pase; EC 3.1.3.9) plays a pivotal role in glycogenolysis and gluconeogenesis catalyzing the last step of both metabolic pathways. Its deficiency leads to glycogen storage disease type Ia (GSD Ia; MIM 232200), which is characterized by hepatomegaly, hypoglycemia, lactic acidemia, hyperuricemia, hyperlipidemia and short stature. The demonstration of a reduced G-6-Pase activity measured in a fresh liver biopsy specimen is still considered the gold standard for verification of the clinical diagnosis of this autosomal recessive disorder. In 1993, the gene (G6PC) spanning 12.5 kb on chromosome 17 and consisting of 5 exons coding for the enzyme was cloned (Lei et al. Science 1993;262:580-3) and mutations were identified. We report the successful molecular genetic analysis of G6PC in 40 patients with GSD Ia. METHODS: DNA was isolated from EDTA-blood, and all 5 exons and the promoter region of G6PC were screened for mutations by SSCP analysis following PCR amplification. Mutations found by SSCP analysis were further characterized by DNA sequencing. In the event of normal SSCP band-patterns, all exons and the promoter region were sequenced. RESULTS: The diagnosis could be verified in all patients. 5 novel mutations, Q20R, W50X, G81R, W156L, and G188D were detected. No true common mutation was identified, with the mutations R83C, Q347X, and G188R occurring in our study population with the highest frequencies of 29%, 15%, and 14% respectively. CONCLUSION: GSD Ia is a genetically heterogenous disease. This study underscores that molecular genetic analysis is a reliable and convenient alternative to the enzyme assay in a fresh liver biopsy specimen to diagnose GSD Ia.

Program Nr: 2792 from the 1999 ASHG Annual Meeting

SOD1 mutation screening in sporadic ALS patients from Russia. *M.I. Shadrina[1], G.N. Levitsky[2], Y.A. Kondratyeva[1], P.A. Slominsky[1], V.I. Skvortsova[2], S.A. Limborska[1]*. 1) Dept Human Genetics, Inst of Molecular Genetics, Moscow, Russia; 2) Dept of Neurology, Moscow Medical University, Moscow, Russia.

Amyotrophic lateral sclerosis (ALS) is degenerative disorder of motor neurons in cortex, brainstem and spinal cord. 10 percent of ALS is familial which inherited as an autosomal dominant trait. It was found, that mutations in gene for Cu/Zn-binding superoxide dismutase (SOD1), in the region 21q22.1-q22.2, accounting for some familial cases of ALS. Mutation in the SOD1 gene can also be responsible for sporadic cases.We observed 15 patients (7 females and 8 males) within age range 29-73 years with idiopathic motor neuron disease and 23 consanguine patient's relatives. 12 patients to had amyotrophic lateral sclerosis, 3 patients had progressive bulbar palsy. We carried out a mutation screening by single stranded conformation polymorphism analysis and direct sequencing of five exons of the SOD 1 gene in these patients. In total, we found mutations in 2 of 15 patients. So, SOD1 gene mutations are infrequent in sporadic ALS patients from Russia. In this patients we identified an aspartic acid to alanine substitution in codon 90 (D90A). One patient is homozygous for the D90A mutation, whereas another patient is heterozygous for this one.

Program Nr: 2793 from the 1999 ASHG Annual Meeting

Novel Mutations and An Update Summary of Mutations in the Glycogen Debranching Enzyme Gene. *W.-L. Shaiu, D.R. Lamson, Y.-T. Chen.* Molecular Genetics, Duke University Medical Center, Durham, NC 27710.

Genetic defects of glycogen debranching enzyme AGL cause glycogen storage disease type III (GSD-III), an autosomal recessive disorder. This multifunctional enzyme has both 1,4-a-D-glucan:1,4-a-D-glucan 4-a-D-glycosyltransferase and amylo-1,6-glucosidase activities, deficiencies of which result in abnormal accumulation of glycogen. Overall loss of AGL activity results in GSD-IIIa, and tissue-specific retention of AGL activity in muscle results in GSD-IIIb. Lack of only glucosidase or transferase activity results in GSD-IIIc or IIId, respectively. We previously described mutations in the coding region of the enzyme that are specifically associated with North African Jewish GSD-IIIa patients (4455delT), or are only found in GSD-IIIb patients (17delAG and Q6X). Here we report the identification in GSD-IIIa patients of a novel class of mutations affecting several 3' and 5' splice sites in the AGL gene. Thus, four individuals were identified with A to G transitions at position -12 upstream of the 3' splice site of intron 32 (IVS 32 A^{-12} ® G) that resulted in insertion of 11 bp intronic sequence between exons 32 and 33. Three of these individuals are heterozygotes and one is the homozygous offspring of non-consanguineous parents. The same mutation has been reported before, although it was associated with a presumed GSD-IIIb patient (Okubo *et al*., Hum Genet 102: 1-5, 1998). Furthermore, we identified a donor splice site mutation at intron 21 (IVS 21 G^{-1} ® A) which caused exon 21 skipping in the mRNA. Other new mutations located in the coding region that resulted in frameshifts and premature terminations also were characterized. Although GSD-IIIa has high genetic heterogeneity, our studies reveal that the molecular defects of GSD-IIIa are clustered disproportionately along the 3' end of the gene, where the putative glycogen binding domains are postulated to reside.

Program Nr: 2794 from the 1999 ASHG Annual Meeting

Common Mutation in Transglutaminase 1 in Lamellar Ichthyosis. *Y.O. Shevchenko, J.G. Compton, J.R. Toro, J.J. DiGiovanna, S.J. Bale.* Genetic Studies Section, NIAMS/NIH, Bethesda, MD.

Lamellar ichthyosis (LI, OMIM#242300) is a severe autosomal recessive skin disorder with an estimated prevalence of 1 in 200,000. LI is characterized by platelike scaling over the entire body, often with a typical presentation at birth of a collodion-like membrane encasing the neonate. Other features of LI include palmar and plantar hyperkeratosis, ectropion, eclabium, and scarring alopecia. Our laboratory established that the disease is caused by mutations in transglutaminase 1 (TGM1), which is located at 14q11 (Russell et al., 1995). The TGM1 gene spans 14 kb and is distributed on 15 exons, whereas the corresponding mRNA is only 2.7 kb in length. Mutations are distributed throughout the TGM1 coding sequence. We have developed an efficient screen for mutations in TGM1 cDNA based on RT-PCR, which can be easily utilized in a clinical laboratory setting. Our analyses revealed a common mutation (29% of US families in our data set) involving loss of the intron 5 splice site acceptor and leading to alternative splicing of the message, which results in full or partial loss of enzyme activity. We have found families in which the splice site mutation was homozygous in affected individuals, as well as families where the patients were compound heterozygotes for the splice site mutation and another TGM1 mutation. A mutation at this same site has been reported to account for the majority of Norwegian LI patients (Pigg et al., 1998). Results of marker typing and haplotype analysis around the TGM1 locus suggested the existence of a founder effect for the mutation. The conserved region spans an interval between markers D14S581 and D14S1032, which are separated by approximately 1.5 cM. Using our set of families that derive from a mix of ethnic and racial backgrounds but have no known Norwegian ancestors, we have traced the founder chromosome to the region of Westfalia in Germany.

Program Nr: 2795 from the 1999 ASHG Annual Meeting

A second family with familial encephalopathy with neuroserpin inclusion bodies (FENIB). *A.E. Shrimpton[1], R.L. Davis[1], P.D. Holohan[1], M.S. Yerby[2].* 1) SUNY Health Sci Ctr, Syracuse, NY; 2) University of Washington School of Medicine, Seattle, WA.

We have previously described a point mutation, S49P, in the neuroserpin gene (PI12), in a large family with familial encephalopathy with neuroserpin inclusion bodies (FENIB). We have called this mutation PI12 Syracuse. A maximum lod score of 3.40 at zero recombination is obtained between PI12 Syracuse and FENIB. The S49P mutation occurs at the homologous serine that leads to a-1 antitrypsin polymerization when substituted by phenylalanine (S53F) as seen in a-1 antitrypsin Siiyama, which leads to inclusion body formation in the liver. A different mutation, S52R, which we have called, PI12 Portland, has been detected in a second family with neuroserpin inclusion bodies (Collins-bodies). The disease in this family, progressive dementia and epilepsy also has an autosomal dominant mode of inheritance but with an earlier onset ages (ages 19 and 30) than that seen in the original family (ages 45 to 55). Affected individuals presented with seizures and a progressive deterioration of personality and memory. The 20 year or so earlier onset associated with PI12 Portland is in keeping with the predicted greater severity of the S52R from modeling of the serpin template. PI12 Syracuse and PI12 Portland are the first examples of neurodegenerative disease resulting from a mutation in a serpin gene. The mutations lead to polymerization of neuroserpin in neurons, which being long-lived non-dividing cells are particularly susceptible to the accumulation of polymerized neuroserpin.

Program Nr: 2796 from the 1999 ASHG Annual Meeting

Mutation analysis in Stargardt disease: Direct sequencing and Southern analysis of *ABCR*. *N.F. Shroyer, R.A. Lewis, J.R. Lupski.* Baylor College of Medicine.

Stargardt disease (STGD) is an autosomal recessive, juvenile onset macular dystrophy which affects 1:10,000 individuals. The STGD locus was mapped to a 2 cM region on chromosome 1p with no evidence for genetic heterogeneity observed in families segregating recessive STGD. The gene causing STGD is a rod photoreceptor specific ATP-binding cassette transporter, *ABCR*. The *ABCR* gene consists of 50 exons encoding an 8 kb, retina-specific transcript. We recently reported a screen for mutations in 150 unrelated STGD patients with heteroduplex and SSCP analysis, that yielded a mutation detection of 57% (173/302 disease chromosomes; *Am J Hum Genet* (1999) 64:422-434). Other investigations using similar methods have yielded mutation detections of 19% (82/430 disease chromosomes), 31% (41/132 disease chromosomes), 32% (35/110 disease chromosomes) 6:291-295) and 62.5% (50/80 disease chromosomes). Thus, many mutations underlying recessive STGD are unidentified by these screening methods. To investigate the frequency of coding region alterations in STGD patients, we initiated direct sequencing of the 50 exons of *ABCR* in 24 unrelated STGD probands. Thus far, we have identified mutations in 25/34 (74%) disease chromosomes in which sequencing is 94% complete. This higher mutation detection rate suggests that some coding region mutations are not detectable by heteroduplex or SSCP analysis and that mutation detection efficiency with these methods ranges from 26% to 84%. To identify the remaining ~25% of STGD-associated *ABCR* mutations, we have initiated a screen for DNA rearrangements with Southern blot analysis. We have selected a cohort of 97 STGD patients in which a PCR-based mutation screen failed to identify one or both mutant alleles. DNA from each patient is then analyzed with six probes encompassing eight to ten exons each; dosage of each band is compared to a standard probe (*osteoprotegerin*, chromosome 8q24). Our data suggest that heteroduplex and SSCP analysis is not sufficient to identify all *ABCR* mutations, and further suggests that some *ABCR* mutations do not result from coding region point mutations.

Program Nr: 2797 from the 1999 ASHG Annual Meeting

Five novel germline gene variants in the RET proto-oncogene identified in MEN type 2 (MEN2) patients. *K. Snow[1], M. Wick[2], R. Mao[1].* 1) Molecular Genetics Lab, Mayo Clinic, Rochester, MN; 2) Department of Laboratory Medicine and Pathology, University of Minnesota, MN.

In 95% of MEN2A patients and in 85% of familial medullary thyroid carcinoma (FMTC) cases, germline mutations occur at one of five cysteine residues in the extracellular domain of the RET proto-oncogene (at codons 609, 611, 618, 620, and 634). Using DNA sequencing to identify mutations within exons 10 and 11, we have identified novel DNA alterations in the RET proto-oncogene in 6 unrelated cases. Case 1 is a 35 year-old male with elevated calcitonin and a history of unilateral pheochromocytoma that was resected; DNA testing showed an alteration at codon 666: AAG>GAG (K666E). Case 2, which is unrelated to case 1, had the same codon 666 alteration. In case 2, testing of multiple family members demonstrated that the alteration segregated with MTC and C-cell hyperplasia. Case 3 is a male with bilateral pheochromocytoma whose maternal maternal great-grandfather was also reported to have had pheochromocytoma; DNA testing identified two alterations: codon 631 GAC>GTC (D631V) and codon 665 CAC>CAG (H665Q). The same alterations were detected in the unaffected mother and unaffected maternal grandmother of case 3. Case 4 is a 37 year-old female with MTC and a family history of thyroid cancer; alterations at codon 634 (TGC>TAC; C634Y) and at codon 631 (GAC>GAA; D631E) were detected. Case 5 is a 68 year-old male with metastatic MTC and no family history of MEN2 or MTC; DNA testing found the alteration 1955-11 G>A in intron 9. Case 6 is a presymptomatic male with a family history of MTC; alterations at codon 618 (TGC>AGC; C618S) and at codon 623 (GAA>AAA; E623K) were detected. Testing of family members of case 6 showed that C618S and E623K were on different chromosomes and that carriers of the E623K alone did not have any clinical manifestations of MEN2 or FMTC. When novel DNA alterations are found, family studies may be helpful in determining clinical significance of findings. However, additional case reports, animal models, and/or functional studies are needed to be certain of the clinical significance of novel variants.

Program Nr: 2798 from the 1999 ASHG Annual Meeting

Characterization of TCOF1 mutations in Brazilian patients affected with Treacher Collins syndrome. *A. Splendore, E.O. Silva, L.G. Alonso, D. Brunoni, N. Alonso, A. Richieri-Costa, D. Cavalcanti, G. Carakushansky, M. Zatz, M.R. Passos-Bueno.* Depto. de Biologia, Univ. de São Paulo, São Paulo, SP, Brazil.

Treacher Collins syndrome (TCS) is a well-characterized autossomal dominant disorder affecting craniofacial development. The estimated incidence is 1/50,000 live births, with 60% of the cases resulting from fresh mutations. Great phenotypic variability is observed within and between families. The gene associated with the syndrome, TCOF1, mapped to chromosome 5q32, was cloned in 1997. In order to characterize TCOF1 mutations in 24 (14 sporadic and 10 familial) unrelated Brazilian patients with a clinical diagnosis of TCS, we screened the 25 coding exons of the gene by SSCP. The exons displaying a mobility shift were sequenced and tested in a 100-chromosome control sample. Thirty-five different mobility shifts were detected by SSCP. Eighteen of these were polymorphisms, including 14 previously undescribed ones. Of the 17 rare alterations that were not detected in the control sample, 11 were new pathogenic alterations, including 7 deletions and 4 insertions, all causing a frameshift. Two other previously described pathogenic mutations were also found: a splice-site alteration and a 5-bp deletion in exon 24. Interestingly, this deletion was found in a much higher proportion among our patients (5/24) as compared with other studies. The 6 remaining rare alterations were silent or intronic, and two of them were also found in unaffected relatives, suggesting that they are not the main cause of the syndrome. Furthermore, 4 patients presented one of these alterations in addition to a pathogenic mutation. The combination of such rare alterations or some of the polymorphisms with a frameshift mutation could be causing the marked phenotypic variability observed in TCS patients The effectiveness of SSCP in detecting the causative mutation in TCS patients is 75%. The 25% (6/24) undetected mutations could be due to lack of sensitivity of SSCP analysis or the presence of mutations in other regions of the gene, as in the promoter or introns. Alternatively, we cannot rule out a heterogeneous nature for TCS.

Program Nr: 2799 from the 1999 ASHG Annual Meeting

Hemochromatosis mutations C282Y and H63D in "cis" phase. *E.L. Spriggs[1,2], P.E. Harris[3], L.G. Best[1,2].* 1) Dept of Clinical Chemistry, Health Sciences Centre, Winnipeg, Manitoba, Canada; 2) Dept of Human Genetics, Univ of Manitoba, Winnipeg, Canada; 3) Faculty of Medicine, Univ of Manitoba, Winnipeg, Canada.

Homozygosity for the C282Y mutation of the HFE gene is a highly significant risk factor for the development of hereditary hemochromatosis (HH) and 83-100% of patients with HH have this genotype. Compound heterozygotes (C282Y/H63D) account for 2-5% of HH patients, and the clinical significance of the H63D mutation in the homozygous state is uncertain. Among 6053 individuals genotyped for these two mutations in 13 studies, no instances of either homozygous C282Y or H63D individuals who also carry the alternate mutation have been reported.

Genotyping was carried out on a 76 year old, Irish/Belgian female with a elevated serum ferritin of 493 ug/L (normal 40-330). She has one sister with a ferritin level of 1056 ug/L, liver disease consistent with HH and bronze skin pigmentation. The affected sister's genotype is currently being determined.

The proband was tested by two PCR amplifications, and the products were digested with SnaBI for C282Y and Bcl I for H63D. Gel electrophoresis of digested products showed the patient to be homozygous for C282Y and heterozygous for H63D. Testing a second sample from the same patient yielded identical results. Concerns that a polymorphism may be interfering with primer binding were addressed by PCR with primers that outflank the original C282Y primers. Results were consistent with the initial genotype. Both the original and second C282Y PCR products were sequenced in both directions, confirming homozygosity for the expected G to A change at nucleotide 845. Sequencing of the H63D product revealed the anticpated heterozygous C to G change at nucleotide 187.

Implications of this finding are: 1) The compound heterozygous state is by far the most common, but not the universal phase for individuals found to be heterozygous for the two mutations. 2) C282Y and H63D in "cis" phase may account for some cases of questionable parentage.

Program Nr: 2800 from the 1999 ASHG Annual Meeting

Human Cytochrome P4501B1: Expression in E. coli and report of 21 new mutations in subjects with Primary Congenital Glaucoma. *I. Stoilov[1], I. Jansson[2], J. Schenkman[2], M. Sarfarazi[1]*. 1) Molecular Ophthalmic Genetics Laboratory, Surgical Research Center, Department of Surgery, and; 2) Department of Pharmacology University of Connecticut Health Center, Farmington, CT.

Molecular genetic studies have already established the existence of a hereditary form of primary congenital glaucoma (PCG) that is caused by mutations in the CYP1B1 gene. During an ongoing screening of PCG probands, we identified a total of 21 new DNA alterations: 16 misense mutations and 5 deletions/insertions. This brought about the number of mutations identified by us to 37 and provided evidence for an extensive allelic heterogeneity. These mutations were observed in 87% of familial and 27% of sporadic cases. The PCG associated with CYP1B1 mutations was inherited as an autosomal recessive trait with 100% penetrance and with both sexes being equally affected. Moreover, there was strong evidence that the sporadic cases were the result of marriages between two normal carriers of CYP1B1 mutations. In parallel to our ongoing mutation screening, the expression study of recombinant human CYP1B1 in E. coli has also been underway. The complete coding sequence of CYP1B1 has been amplified from retinal cDNA library and subcloned into pCWori+ expression vector. Degenerate PCR amplifications were used to create a series of modified expression constructs. The modifications were directed toward increasing the AT content at the 5'-end of the wild type sequence. The different constructs were introduced into DH5a competent cells. Hemoprotein expression was quantified by difference spectroscopy. An efficient construct serving as a template for In Vitro mutagenesis experiment was selected to introduce ten different CYP1B1 mutations in the wild type sequence. These are a series of missense mutations previously reported by us that affect key structural elements of either the hinge region or the conserved core structures of CYP1B1. This expression system is currently being used to produce both wild type and mutant forms of CYP1B1, which will serve as a tool for comparative functional analysis. Support: EY-11095, Glaucoma Foundation and InSite Vision, Inc.

Program Nr: 2801 from the 1999 ASHG Annual Meeting

Silent codon substitution in the VHL gene results in exon skipping and familial pheochromocytoma. *C.A. Stolle[1], V. Kasparcova[1], G.J. Tsongalis[2], S. Inzucchi[3].* 1) Dept Genetics, Univ Pennsylvania Sch Medicine, Philadelphia, PA; 2) Dept Pathology, Hartford Hospital, Hartford, CT; 3) Section of Endocrinology, Yale U School of Medicine, New Haven, CT.

Von Hippel-Lindau disease is an autosomal dominant cancer predisposition syndrome due to mutations in the VHL gene and characterized by hemangioblastoma, retinal angioma, clear cell renal carcinoma, pheochromocytoma, neuroendocrine tumors and cysts of the pancreas, endolymphatic sac tumors, and papillary cystadenomas of the epididymis and broad ligament. Pheochromocytomas occur in some VHL families as the predominant or only characteristic tumor. In 98% of cases of VHL with pheochromocytomas, the gene defect is a missense mutation, suggesting that an intact, but defective protein is necessary for development of adrenal tumors. We have followed a three generation family with pheochromocytoma but no other overt manifestations of VHL. DNA sequence analysis revealed a silent mutation in codon 138 (A to G nt 627) that segregated with the disease in this family and was not seen in over 100 normal alleles. To determine whether this mutation could alter splicing of the primary transcript, total RNA from the proband and an unaffected control was reverse transcribed and amplified with primers specific for VHL exons 1 and 3. Two bands corresponding to alternatively spliced transcripts characterized by inclusion (isoform I) or exclusion (isoform II) of exon 2 were present in both the proband and control samples. However, the percentage of isoform II was greater than 50% in the proband, but less than 20% in control samples. This mutation, although outside the consensus splice sites, apparently causes efficient skipping of exon 2, resulting in the in frame removal of 41 amino acids. The data support the observation that a defective, but not necessarily full-length protein predisposes to pheochromocytoma. Furthermore, the fact that a silent mutation in the VHL gene can cause disease suggests that functional assays may be needed to rule out the disease-causing potential of some apparently neutral polymorphisms.

Program Nr: 2802 from the 1999 ASHG Annual Meeting

Linkage and Candidate Gene Analysis of Two Pedigrees with Charcot-Marie-Tooth Neuropathy Type 1C. *V.A. Street[1], M.P. Keller[2], R.A. George[2], A.S. Golden[1], B.L. Tempel[1], T.D. Bird[3], P.F. Chance[2].* 1) Otolaryngology and Bloedel Hearing Research Center, University of Washington, Seattle, WA; 2) Pediatrics, Division of Genetics and Development, University of Washington, 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 three 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 2 gene (EGR2) also known as Krox-20, 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.

Program Nr: 2803 from the 1999 ASHG Annual Meeting

Molecular heterogeneity of Carbonic Anhydrase II deficiency in Italy. *P. Strisciuglio[1], G. Bonapace[1], D. Concolino[1], G. Ruffa[2], A. Dammacco[3].* 1) Dept. of Pediatrics, University of Catanzaro, Italy, MD; 2) Dept. of Pediatrics, University of Genoa, Italy, MD; 3) Hospital "Giovanni XXIII" of Bari, Italy, MD.

Deficiency of Carbonic Anhydrase (CA II) results in a syndrome of osteopetrosis, renal tubular acidosis and cerebral calcification, that is inherited as an autosomal recessive disorder. Other features include mental retardation, frequent fractures, growth failure and dental malocclusion. The geographical distribution of this disease is striking, with more than half the known case observed in families from mediterranean area. In the last years, we have identified several families affected by CA II deficiency. All the families, except one, came from Southern Italy. We performed molecular analysis on the patients by SSCP and direct sequencing. Interestingly, the first family discovered turned to have the same missense mutation Hys107Tyr found in a homozygous belgian patient and in three american siblings of italian origin who were compound heterozygotes. Moreover, a single base pair deletion in exon 2 was identified in the family of Northern Italy and a splice junction mutation at the 5' of intron 6 was found in another family in whom we performed the first prenatal diagnosis. We extended the molecular analysis to two new families coming from Southern Italy. The patient of the first family turned to be homozygous for the missense mutation Hys107Tyr. The brothers of the second family were heterozygous for an insertion gt of two bases in exon 6, undescribed previously, and a polimorphism CTG-TTG with an unchanged aminoacid (Leu) at the same exon 6 was present. Studies are in progress to complete the characterization of this family. Our results show that our families are different from arabic patients accounting for 75% of reported cases of CA II deficiency in whom a splice junction mutation at the 5' of intron 2 is present. Also a frame shift mutation in exon 7 is the most common mutation in hispanic patients from the Caribbean Islands. In conclusion, our data underline the presence of a novel mutation in one of our families and suggest a molecular heterogeneity of CA II deficiency in Italy. A genotype-phenotype correlation in not possible at least for italian patient.

Program Nr: 2804 from the 1999 ASHG Annual Meeting

Cloning, characterization and mutation screening of the RP1 gene in autosomal dominant retinitis pigmentosa.

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We recently identified a novel retina-specific gene as the cause of the RP1 form of adRP. The RP1 gene codes for an unusually large protein (2,156 amino acids) with a relatively short region of sequence similarity to the doublecortin family of proteins. We have also identified another member of this gene family, apparently retina-specific, which maps to the p-arm of chromosome 8.

In mouse, the RP1 gene is expressed predominantly in photoreceptors and expression occurs primarily during postnatal development. We have mapped the mouse RP1 gene to mouse chromosome 1, near the centromere, using two different techniques. This localization excludes RP1 as the causative gene for the mouse retinal degeneration *Rd4*, previously thought to be the mouse model for RP1.

Mutation screening of RP1 in over 250 adRP patients shows that the frequency of RP1 mutations is at least 6% and potentially higher, since screening is not complete in all patients. All disease-causing mutations identified so far are nonsense or frameshift mutations, causing premature truncation of the protein. These mutations cluster in a region of the protein beyond the doublecortin domain. Two potential missense mutations have also been found in adRP patients but it is not yet clear if they are disease-causing. A number of highly polymorphic amino acid substitutions have been identified, indicating that the protein has numerous common isoforms. Comparison to the mouse protein sequence reveals both highly conserved and highly divergent domains, the former probably representing important functional domains.

Supported by NEI-NIH grant EY07142 and grants from the Foundation Fighting Blindness, the Hermann Eye Fund, the March of Dimes Found., the British RP Soc. and the Wellcome Trust.

Program Nr: 2805 from the 1999 ASHG Annual Meeting

Nonsense mutation of the actinin 3 gene is not associated with severity of dystrophinopathy. *R. Suminaga[1], Y. Takeshima[1], H. Wada[3], H. Nakamura[1], M. Matsuo[2].* 1) Pediatrics, Kobe Univ. School of Medicine, Kobe, Hyogo, Japan; 2) Division of Genetics, ICMR, Kobe Univ. School of Medicine, Kobe, Hyogo, Japan; 3) Sakura Ryoikuen Hospital, Sanda, Hyogo, Japan.

There is a wide variety of clinical severities among patients with Duchenne or Becker (DMD and BMD) muscular dystrophy especially in the wheelchair bound age. To explain this, some modifying factors are supposed to be present in addition to the mutations in the dystrophin gene. Recently, a common nonsense mutation causing alpha actinin 3 deficiency was reported. Considering that alpha actinin 3 is expressed specifically in type 2 muscle fibers and that the alpha actinins are a family of actin-binding and crosslinking proteins related to dystrophin, we supposed that nonsense mutations of the alpha actinin gene function as modifying factors for the clinical phenotype of DMD/BMD. Frequently detected in the ACTN3 gene is a C to T transversion at position 1747 in exon 16 which converts arginine (CGA) to a stop codon (TGA) thus creating a novel Ddel restriction site. We, therefore, analyzed the genotype of the ACTN3 gene in 150 Japanese DMD/BMD cases and 27 normal Japanese controls by PCR amplification of the genomic fragment between exons 15 and 16. This amplified fragment then subsequently underwent digestion using Ddel. Homozygosity for the stop codon was identified in 32 of 150 (21.3%) of the DMD/BMD patients and in 7 of the 27 (25.9%) Japanese controls. No significant statistical difference was found when these two values were compared. Of the 76 DMD cases, 12 (15.8%) were homozygous and 42 (55.3%) were heterozygous for the nonsense mutation and no significant difference was noted with regards the age when these DMD patients first started walking nor with regards the age when they became wheelchair bound. These results suggest that no association exists between the nonsense mutation of the alpha actinin gene and the clinical phenotype of DMD/BMD.

Program Nr: 2806 from the 1999 ASHG Annual Meeting

FMR1 mRNA expression levels in fragile X. *F. Tassone[1], RJ. Hagerman[2], AK. Taylor[3], JB. Mills[1], S. Wood[2], LW. Gane[2], PJ. Hagerman[1]*. 1) Biochemistry and Molecular Gen, UCHSC, Denver, CO; 2) 2 Child Development Unit, The Children's Hospital, Denver, CO; 3) 3 Kimball Genetics, Inc., Denver, CO.

Premutation carriers are generally believed to be clinically unaffected, their alleles unmethylated and associated with normal transcription and normal FMRP production. However, some individual carriers of the FMR1 premutation show a broad variety of psychopathological symptoms including learning disabilities, emotional problems and in some cases mental retardation. In order to establish whether the observed differences in phenotypic involvement among premutation individuals may be associated at molecular level with FMR1 gene dysfunction, we have investigated the FMR1 mRNA expression levels by quantitative fluorescence RT-PCR, and FMRP expression by immunocytochemistry in 40 premutation carriers (27 females and 13 males). Unexpectedly, we found that all male carriers (5) of a premutation above 110 CGGrepeats and presenting clinical features typical of fragile X syndrome, showed a substantial increase in the FMR1 mRNA. In addition, 3 of the male carriers showed a significant decrease of FMRP expression detected by immunocytochemistry. These data suggest that the fragile X phenotype does not always correlate with a decrease in FMR-1 mRNA and that the absence or deficit of FMRP expression does not arise only from decreased mRNA levels. Thus, other mechanisms may be involved in the regulation of the FMR-1 transcription. Results will be discussed.

Program Nr: 2807 from the 1999 ASHG Annual Meeting

Homologous and non-homologous recombinations at the glucocerebrosidase locus: Implications for Gaucher disease. *N. Tayebi, B. Stubblefield, D. Stone, M. Callahan, V. Madike, E. Sidransky.* DHHS/PHS, NSB/NIMH/NIH, Bethesda, MD.

The molecular diagnosis of Gaucher disease, the inherited deficiency of the lysosomal enzyme glucocerebrosidase (GC), is complicated by the presence of a 96% homologous pseudogene, which is located 16kb downstream of the functional gene. Metaxin, a convergently transcribed gene adjacent to the GC pseudogene, also has a highly homologous pseudogene downstream of the GC gene. Several patients with Gaucher disease have mutant alleles which have resulted from recombination between these two genes and their pseudogenes. Earlier studies of genotype-phenotype correlations revealed that Gaucher disease patients with diverse clinical manifestations can have the same point mutations. However, some of these point mutations, particularly L444P, originate from the pseudogene sequence and are often accompanied by other pseudogene sequence changes, suggesting the presence of recombination. We studied 398 alleles from 199 patients with Gaucher disease and identified 40 recombinant alleles (17 Type 1, 16 Type 2, and 7 Type 3). The sites and the possible mechanisms of crossover were determined using several methods. Southern blots of genomic DNA digested with the restriction enzymes SstII and SspI demonstrated major rearrangements. These included gene fusion with the deletion of the intergenic region in 13 alleles and a duplication in three alleles. Southern blots of HincII digested DNA, long template PCR, and direct sequencing identified 24 additional recombinant alleles. Sequencing identified eight different crossover sites and indicated the probable crossover site in many other alleles. In 75% of recombinant alleles, the crossover occurred within introns 8-11. Different mechanisms, including reciprocal and nonreciprocal recombination, intramolecular crossover, and chi structures appear to have occurred in different cases. One possible motif sequence, which could be a hot spot for crossover, was identified. Homozygosity for recombinant alleles appears to result in early lethality. Further studies of these recombinant alleles may provide insights into the etiology of the more atypical Gaucher phenotypes.

Program Nr: 2808 from the 1999 ASHG Annual Meeting

CTNS MUTATIONS IN AN ITALIAN POPULATION OF CYSTINOSIS PATIENTS. *R. Tenconi[1], S. Mason[1], M. Clementi[1], R. Dall'Amico[2], G. Zacchello[2], G. Ardissino[2], C. Pecoraro[2], A. Burlina[2], E. Zamorani[2], F. Ginevri[2], M. Pennesi[2], P. Sorino[2]*. 1) Dept Ped, Clinical Genetics, Univ Padova, Padova, Italy; 2) Italian Registry of Cystinosis.

Nephropatic Cystinosis (NC) is an autosomal recessive lysosomal storage disease (OMIM 219800) characterized by renal failure in pediatric age and other systemic complications. The cystinosis gene was mapped to chromosome 17p13 in 1995 and cloned in 1998. Two studies performed in European and American patients for a total of 178 subjects have found 33-44% of patients to be homozigous for a 65-Kb deletion encompassing a polymorphic marker (D17S829) located within the intron 3. Several mutations have been found in the remaining patients, while no mutations were identified in about 20% of subjects. We have studied 17 probands from the Italian Registry of NC and found 2/17 homozigous and 3/17 heterozygotes for the 65-kb deletion. The analysis by SSCP of the exon 7, where a recurrent nonsense mutation (G--A substitution) was found in European patients, did not identify any mutation in our patients. We present the mutational spectrum of CN in Italy.

Program Nr: 2809 from the 1999 ASHG Annual Meeting

Creation and characterization of transgenic mouse lines expressing Cre recombinase in skeletal muscle or neurons. *F. Tiziano, P. Miniou, T. Frugier, A. Dierich, M. Le Meur, J. Melki.* IGBMC. INSERM/CNRS/ULP, Illkirch, Strasbourg, France.

In order to elucidate the pathophysiology of spinal muscular atrophy (SMA), a conditional mutagenesis of the mouse SMN gene has been undertaken using the CRE-LoxP system. We inserted two loxP sites flanking SMN exon 7 (SMNF7) through homologous recombination in order to delete SMN exon 7, the most frequent mutation found in SMA. Since homozygous deletion of SMN exon 7 in all cell types results in early lethality of mouse embryos, our goal was to restrict the induced mutation to either skeletal muscle or neurons, the target cells in SMA. For this purpose, we generated transgenic mouse lines expressing Cre recombinase under the control of the human alpha-skeletal actin (HSA-Cre) or rat neuron specific enolase (NSE-Cre) promoters. To test the efficiency of Cre recombinase activity, HSA-Cre transgenic lines were crossed with LacZ reporter line (Akagi et al, 1997). Specific recombinase activity was demonstrated in heart and skeletal muscle, in a pattern similar to the expression of the endogenous alpha-skeletal actin gene, both in adult tissues and during development. To test Cre recombinase activity in NSE-Cre transgenic lines, transgenic mice were crossed with (SMNF7/+) mice, and double transgenic mice were selected. Cre mediated deletion of SMN exon 7 was quantified by both Southern blot and semi-quantitative PCR amplification analysis of genomic DNA extracted from various tissues. The extent of induced deletion was variable, ranging from very low percentage in heart or skeletal muscle, to 47% in spinal cord and 56% in brain. In these tissues, the intermediate values may reflect Cre recombinase activity restricted to certain cell types. In order to further characterize tissue-specificity of Cre recombinase expression, in situ hybridization using Cre riboprobe is currently performed. The reported transgenic lines can be used to target SMN exon 7 deletion to either skeletal muscle or neurons. Therefore, this approach may avoid embryonic lethality resulting from inactivation of the SMN gene and should allow to create an animal model of SMA.

Program Nr: 2810 from the 1999 ASHG Annual Meeting

Mendelian Cytogenetics Network. A resource for genotype-phenotype delineation in man. *N. Tommerup[1], M. Bugge[1], K. Brondum-Nielsen[2], U. Kristoffersson[3], P.K.A. Jensen[4], H.H. Ropers[5]*. 1) Dept. of Med. Genet., Univ. of Copenhagen, Denmark; 2) J.F. Kennedy Inst., Glostrup, Denmark; 3) Dept. of Clinical Genet., Univ. Hosp. of Lund, Sweden; 4) Dept. of Clinical Genet., Univ. Hosp. of Aarhus, Denmark; 5) Max-Planck-Inst. for Mol. Genet., Berlin (Dahlem), Germany.

One evident link between phenotype and genotype are disease-associated balanced chromosome rearrangements (DBCRs) that truncate/inactivate specific genes. We have established a network of cytogenetic laboratories, Mendelian Cytogenetics Network (MCN), for the systematic identification and mapping of DBCRs. This includes an online database MCNdb (http://mcndb.imbg.ku.dk) and a YAC panel for FISH mapping of the breakpoints (http://www.mpimg-berlin-dahlem.mpg.de/~cytogen/). To get an estimate of the potential of this approach, we surveyed the cytogenetic files in Denmark/Scania, with a population of ~7 million. The 8 labs involved have made ~70.000 postanatal cytogenetic tests since 1970. We identified 210 DBCRs, excl. Robertsonians and the common chr. 9 inversion. The frequency of DBCRs (0.3%) is two-three times more frequent than in the general population, suggesting that up to 2/3 of the cases may be causally related to the traits involved. Of the 210 cases identified, only 10% have been published previously. Several of the unpublished DBCRs immediately defined specific/promising breakpoints associated with Nail-patella, Prader-Willi, Rett and Schmidt syndromes, Crohns disease, VATER association, ataxia, and ulna-aplasia. The remaining DBCRs were associated with mental retardation, autism, dysmorphic features, and male and female infertility. The implications of the survey is apparent when we compare with the size of MCN: Altogether, the 292 participating cytogenetic laboratories have performed >2.5 million postnatal analyses, with an estimated ~7.500 DBCRs stored in the cytogenetic files, of which 2/3 (~5.000) might be causative mutations. In addition, an estimated ~450 novel cases should be added each year. Our data illustrate that DBCRs and MCN are resources for large scale establishment of phenotype-genotype relationships in man.

Program Nr: 2811 from the 1999 ASHG Annual Meeting

Mohr-Tranebjaerg Syndrome is an X-linked Recessive Disorder Characterized by Mitochondrial Dysfunction Associated with Neuronal Cell Death. *L.. Tranebjaerg[1], S. Lindal[1], S. Merchant[2], OC. Ingebretsen[1], B. Hamel[3], V. Fung[4], M. Hayes[4], C. Koehler[5], O. Nilssen[1], M. van Ghelue[1]*. 1) Dept Medical Genetics, Dept Pathol, Dept Clinical Chemistry University Hosp, Tromsoe, Norway; 2) Temporal Bone Registry, Massachusets Gen Hosp, Boston; 3) Dept Human Genet Univ Hosp Nijmegen,Netherlands; 4) Dept Neurol,Westmead Hosp, Westmead, Australia; 5) Biozentrum Basel Univ, Basel, Switzerland.

Mohr Tranebjaerg syndrome (MIM 304700) is clinically well characterized and shown to be due to frameshift /stop mutations in the DDP gene(Tranebjaerg L et al, J Med Genet 32; 257-263, 1995; Jin H et al, Nat Genet 14;177-180,1996).We present the updated spectrum of mutations in 7 families identified in Norway,USA,Spain,Denmark, Australia,and the Netherlands each having a private DDP mutation. Recently,a DDP homologue(TIM 8)in yeast was shown to be involved in mitochondrial membrane protein transport (Koehler C et al,PNAS; 99:2141-6,1999)which implies that human deafness-dystonia syndrome is likely to be a mitochondrial dysfunction associated with neurodegenerative disease. Restudy of patients from the original Norwegian family showed optic atrophy,loss of neurons in the basal ganglia, morphologically abnormal mitochondria in muscle EM analysis and reduced number of spiral ganglion cells at the analysis of temporal bone.The DDP protein was absent in cultured fibroblasts as determined by means of polyclonal antibodies.Our findings for the first time support mitochondrial dysfunction in human patients with deafness-optic atrophy-dystonia syndrome.The ATP production rate in fresh muscle biopsy specimens was slightly reduced.There are striking clinical and neuropathological similarities between this disorder and other entities caused by mutations in nuclear genes such as Friedreich ataxia, providing independently confirmatory evidence that gene mutations causing mitochondrial dysfunction have a predilection for compromizing the functions of regions of the central nervous system requiring high metabolic turn over,such as the auditory and visual pathways.Financial support is acknowledged from the Norwegian Research Council(grant no 114575/320).

Program Nr: 2812 from the 1999 ASHG Annual Meeting

Identification of a new COL2A1 mutation in a family with autosomal dominant Spondyloepimetaphyseal dysplasia (SEMD) Strudwick type. *C.J. Tysoe[1], J. Saunders[1], L. White[1], N. Hills[1], G. Evans[2], T. Cole[3], S. Chapman[4], A. Richards[5], S. Martin[5], M. Pope[1]*. 1) Institute of Medical Genetics, Uni Wales College of Medicine, Cardiff, U.K; 2) Robert Jones and Agnes Hunt Orthopaedic and District Hospital NHS Trust, Oswestry, Shropshire, SY10 7AG; 3) West Midlands Regional Clinical Genetics Service, Clinical Genetics Unit, Birmingham Womens Hospital, Edgbaston, Birmingham, B15 2TG; 4) Birmingham Childrens Hospital, Steelhouse Lane, Birmingham, B4 6NH; 5) MRC Connective Tissue Genetics Group, Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge.

We have carried out mutation screening analysis of the COL2A1 gene in a three generation family with an autosomal dominant form of Spondyloepimetaphyseal dysplasia (SEMD) Strudwick type. SEMD is a specific member of a clinically heterogeneous group of skeletal disorders, characterised by defective growth and modelling of the spine and long bones. Common clinical features include disproportionate short stature, malformed vertebrae and abnormal epiphyses or metaphyses. The family exhibited specific phenotypic features including chest deformity, characteristic limb abnormalities, myopia and early-onset degenerative joint disease; specifically, the proband showed limb shortening, abnormal epiphyses and metaphyses with tilting of the left tibial epiphysis. Direct sequencing of PCR amplified genomic DNA showed a point mutation within exon 20 of the COL2A1 gene, causing a glycine to aspartic acid substitution at codon 261. Previous reports have identified five point mutations causative for SEMD (three in SEMD Strudwick patients and two in unclassified SEMD patients, who exhibited some characteristics of SEMD Strudwick type). All five point mutations resulted in the substitution of a glycine residue for an alternative amino acid. Here we report the finding of a sixth point mutation, also a glycine substitution, which provides additional information as to the mutational spectrum of this disorder, with a view to further defining the genotype/phenotype correlation for this specific skeletal defect.

Program Nr: 2813 from the 1999 ASHG Annual Meeting

Identification of sixteen new PHEX gene mutations in Finnish patients with X-linked hypophosphatemic rickets.

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X-linked dominant hypophosphatemic rickets (HYP) is the most common form of hereditary rickets. It is characterized by a defect in renal phosphate reabsorption and an abnormal 1,25-dihydroxyvitamin D metabolism. Affected patients have short stature, skeletal pain and deformities, and periapical abscesses. The defective gene, PHEX, has been cloned and over 80 mutations identified. Since several other disorders with similar defects in renal phosphate transport and bone mineralization have been described, we have carried out a mutational screening of the PHEX gene to confirm the diagnosis in the Finnish patients. All 22 exons were analysed by SSCP and the aberrantly migrating bands sequenced. Included in the study were 15 sporadic patients and 5 familial cases. We identified 18 mutations, of which 16 were novel. Two families were segregating the same nonsense mutation in exon1. This mutation has been reported in three independent studies previously, and therefore, it presents a mutational hotspot rather than a Finnish founder mutation. Mutations were found in 100% of the familial cases and 94% of the sporadic patients. No apparent genotype-phenotype correlations could be observed. X-inactivation status of the female patients was normal.

Program Nr: 2814 from the 1999 ASHG Annual Meeting

Unusual disease mechanism in a new mutation FSHD family. *M. Upadhyaya[1], T. Haaf[2], M. Osborn[1], P. Thompson[1], S. van der Maarell[3], M. Rogers[1], D. Cooper[1]*. 1) Dept Medical Genetics, Univ Wales, Cardiff, Wales,UK; 2) Max-Planck-Institute,Berlin,Germany; 3) MGC Dept of Human Genetics,Leiden,Netherlands.

Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant muscular disorder. The disease locus has been mapped to 4q35 but the FSHD gene has not yet been isolated. FSHD is causally related to the presence of a shorter than normal EcoRI/BlnI fragment detected with probe p13E-11. This fragment size is <35 kb in FSHD patients. We have recently studied a sporadic FSHD family in whom the male proband exhibited symptoms at age 10 and was wheel-chair bound by age 33. Restriction analysis of DNA from this family following EcoRI and BlnI double digestion and electrophoresis, demonstrated that a 48kb EcoR1/Bln1 fragment was the only band present in the affected child, while both his parents possessed larger (>50kb) DNA fragments. Pulsed field gel electrophoresis analysis with EcoRI/BlnI,demonstrated that the mother was apparently homozygous for a 100kb fragment, whereas the father had two separate fragments of 60kb and 70kb. The affected child had inherited a 60kb paternal fragment but not a 100kb maternal allele. The apparent homozygosity for the 100kb EcoR1/Bln1 fragment in the mother may be explicable in terms of hemizygosity for a chromosome 4 with the affected child inheriting this deleted (null) allele. Further genotype analysis excluded non-paternity or sample mix-up. Analysis with chromosome 4 and 10 telomeric YAC clones indicated that this family is not deleted for the subtelomeric region whilst chromosome-specific painting probes for chromosomes 4 and 10 did not detect any gross rearrangements. FISH studies with a 4.1 kb KpnI-NaeI probe(which encompasses p13E-11 and lacks most of the D4Z4 sequence) suggests a partial deletion in one of the son's chromosome 4 homologue. We are currently defining the size of this deletion.The signal with chromosome 10 was similar in all three members of this family. Sequence analysis of entire FRG1 transcript did not identify any mutations. Thus despite extensive molecular and cytogenetic analysis, the underlying FSHD disease mechanism in this family remains uncertain.

Program Nr: 2815 from the 1999 ASHG Annual Meeting

Partial a-**sarcoglycan (**a-**SG) deficiency associated with the retention of the SG complex in a LGMD2D family.** *M. Vainzof[1,2], E.S. Moreira[1], M. Canovas[1], O.T. Suzuki[1], R.C.M. Pavanello[1], M.R. Passos-Bueno[1], M. Zatz[1].* 1) Dept Neurology, FMUSP; 2) Dept. of Biology, IBUSP, Sao Paulo, Brazil.

Mutations in four genes at 17q, 4q, 13q and 5q, which encodes a-SG, b-SG, g-SG and d-SG are responsible for sarcoglycanopathies, a subgroup of Limb-Girdle muscular dystrophies (LGMD2C-2F). The sarcoglycan proteins are known to be tightly associated with each other and a common immunohistochemical finding in sarcoglycan deficient patients is the loss or a significant reduction of all SG proteins at the sarcolemma, demonstrating that a primary defect in one of the associated glycoproteins results in secondary deficiency of the others components. Here we are describing a Brazilian family, in which the proband showed normal dystrophin and a partial deficiency of a-SG protein but retention of b-, g-, and d-SG in the sarcolemma of muscle fibers. The clinical course of the 3 affected brothers is variable. The oldest, currently 27 years old, was wheelchair bound at age 18. The second showed the first signs at age 11, weakness in upper limbs at age 19 and is still walking at age 23. The youngest, aged 16, reported onset at age 9 with toe walking and frequent falls and is still ambulant. DNA analysis detected a novel mutation in the a-SG gene, a G724T transversion in exon 6, a region close to the transmembrane domain of the protein that probably does not alter the domain of interaction among a-SG and the other SG proteins within the DGC complex. In a recent study on the proportion of SGpathies in the Brazilian population we verified that 23 among 115 LGMD patients showed a deficiency of a-SG, as well as a deficiency of the whole SG complex. However, in 12 other patients, a partial deficiency of only a-SG was detected and a SGpathy was excluded. The present observation suggests that these 12 patients, which represent about 10% of our LGMD patients, might have been misdiagnosed. This result highlight the importance of better investigating patients with a partial or isolated deficiency of any one of the SG proteins. Supported by FAPESP, PRONEX, CNPq, FFM.

Program Nr: 2816 from the 1999 ASHG Annual Meeting

Novel mutations in the BIG-H3 gene associated with an atypical granular corneal dystrophy and lattice corneal dystrophy type IIIA.*BIG-H3*. *S. Valleix[1], P. Dighiero[2], S. Drunat[1], F. D'Hermis[2], J.M. Legeais[2], G. Renard[2], G. Grateau[1], M. Delpech[1]*. 1) Genetique Moleculaire Humaine, Faculte de Medecine, Cochin, Paris, France; 2) Department of Ophthalmology, Hotel-Dieu Hospital, Paris, France.

The BIG-H3 gene, encoding kerato-epithelin, has been previously shown to be the disease gene for, at least, six distinct autosomal dominant corneal dystrophies. A clinical, histological, ultrastructural and molecular analysis of three unrelated French families was performed to determine the mutational status of the BIG-H3 gene. Two of these families were affected with a lattice corneal dystrophy closely resembling type IIIA, while the other one was affected with an atypical corneal dystrophy of granular type. Exons of the BIG-H3 gene were amplified by polymerase chain reaction and analyzed by a direct sequencing method. We identified, in all patients from the two families affected with the lattice dystrophy, a heterozygous single base-pair transition from G to A of the first nucleotide of codon 546 (GCC/ACC) predicting the replacement of alanine by threonine (A546T). This novel A546T mutation identified in these French patients with lattice dystrophy type IIIA differs from that reported in Japanese patients (P501T). Molecular data from patients affected with the unusual form of granular corneal dystrophy revealed a molecular defect never reported as yet. All patients (nine individuals across three generations) were found to be heterozygous for two mutations, the R124L mutation and a novel mutation predicting the deletion of two amino acid residues, threonine (T) and glutamic acid (E), at codons 125-126. These two mutations were both transmitted on the same chromosome in all nine patients with complete penetrance. This molecular defect is intriguing since the presence of R124L mutation alone is responsible for the superficial variant of granular dystrophy, while its association with deletion of codons 125 and 126 produces significant phenotypic variations of granular corneal dystrophy.

Program Nr: 2817 from the 1999 ASHG Annual Meeting

The COCH P51S mutation, a frequent cause of cochleovestibular dysfunction in Belgium and The Netherlands, is present on a common chromosomal haplotype. *G. Van Camp[1], E. Fransen[1], M. Verstreken[1], W.I.M. Verhagen[2], S. Bom[3], C. Cremers[3], F. Cremers[4].* 1) Dept Medical Genetics, Univ Antwerp - UIA, Antwerp, Belgium; 2) Department of Neurology, Canisius-Wilhelmina Hospital, Nijmegen, The Netherlands; 3) ENT Department, University Hospital Nijmegen, The Netherlands; 4) Department of Human Genetics, University Hospital Nijmegen, The Netherlands.

Among the loci for autosomal dominant hearing impairment, the DFNA9 locus on chromosome 14 is the only one involving vestibular problems in addition to non-syndromic deafness. Affected family members typically suffer from progressive hearing loss associated with progressive vestibular problems, such as instability in the dark and vertigo. The gene responsible for DFNA9 is the COCH gene, probably encoding an inner ear specific extracellular matrix protein. A missense mutation, changing a highly conserved Proline into a Serine (P51S), has recently been found in several families in Belgium and the Netherlands. In this study, we have analyzed additional patients and families with cochleo-vestibular dysfunction for the P51S mutation, and determined the haplotype of these families for a number of closely linked markers. In total, we have now identified 10 independent families with the P51S mutation, all living in Belgium and The Netherlands. The haplotype analysis showed a significant amount of allele sharing for all families, indicating that the P51S mutation originates from a common ancestor.

Program Nr: 2818 from the 1999 ASHG Annual Meeting

Skewed X-chromosome inactivation in two manifesting carriers of choroideremia. *J.A.J.M. van den Hurk[1], T.J.R. van de Pol[1], M.A. van Driel[1], L.I. van den Born[2], M. Seeliger[3], B. Wissinger[3], H.-H. Ropers[1,4], F.P.M. Cremers[1].* 1) Department of Human Genetics, University Hospital Nijmegen, Nijmegen, The Netherlands; 2) Institute of Ophthalmology, Erasmus University, Rotterdam, The Netherlands; 3) Universitäts-Augenklinik, Eberhard-Karls-Universität, Tübingen, Germany; 4) Max Planck Institute for Molecular Genetics, Berlin, Germany.

Choroideremia (CHM)is an X-linked recessive eye disorder that is characterized by progressive degeneration of the choroid, retinal pigment epithelium (RPE), and retina. Affected males develop night-blindness in their teenage years, followed by progressive constriction of visual fields and, eventually, complete blindness. Female carriers generally show no serious visual impairment, but they have patchy pigmentation and degeneration of the RPE. There are occasional reports of female carriers who are fully affected by choroideremia. Two of these symptomatic females were found to have X-autosome translocations that involve the *CHM/REP-1* gene.

DNA of two previously undescribed females with clinical signs of choroideremia was searched for *CHM/REP-1* gene mutations. In one female patient exon 1 of the *CHM/REP-1* gene was found to be deleted, the other had a nonsense mutation in *CHM/REP-1* exon 14 (Cys575Stop). In both patients no additional mutations could be detected.

Subsequently, the X-inactivation pattern in lymphocytes of the manifesting carriers was studied by PCR analysis of a polymorphic CAG repeat in the androgen receptor (ARA) gene. One of the females was found to be homozygous for the CAG repeat. X-chromosome inactivation in this patient was studied by Southern blot analysis using probe M27b (DXS255). In both manifesting carriers the normal X-chromosome was found to be inactive in the majority of cells.

In conclusion, the clinical manifestation of choroideremia in the two carrier females is likely to be the result of a *CHM/REP-1* gene mutation and skewed X-chromosome inactivation.

Program Nr: 2819 from the 1999 ASHG Annual Meeting

Niemann-Pick C disease: mutational spectrum in *NPC1* gene and genotype/ phenotype correlations. *M.T. Vanier[1], G. Millat[1], C. Marçais[1], M. Rafi[2], T. Yamamoto[3], J.A. Morris[4], P.G. Pentchev[4], E. Nanba[3], D.A. Wenger[2].* 1) INSERM U189, Lyon-Sud Medical School, Oullins, France; 2) Dpt Neurology, Jefferson Medical College, Philadelphia, PA, USA; 3) Gene Res. Center,Tottori University Medical School, Yonago, Japan; 4) DMNB, NINDS, NIH, Bethesda, MD, USA.

Niemann-Pick C (NPC) disease is a neurovisceral lysosomal lipid storage disorder resulting from mutations affecting the *NPC1* gene in 95% of the families. A recent study of 20 NPC1 patients led to the identification of 20 point mutations, 1bp and 163bp deletions, 1 approx. 500 bp insertion and a 14 bp duplication. The mutations were distributed throughout the gene, with 8 of them located before the region coding for the sterol sensing domain (SSD) (first 11 exons), 3 within that region, 13 in the last 10 exons. They were all private apart from one important exception, the T3182C transition, which leads to a I1061T substitution affecting a transmembrane domain. In a further study, I1061T was shown to be the most frequent mutation in NPC patients of Western European descent (including in USA and Canada)(40/290 mutant alleles) and from the study of 14 homozygous patients, to correlate with a classical juvenile phenotype. This mutation was demonstrated as highly prevalent in the described isolate of Hispanic NPC patients from the upper Rio Grande valley of the USA. Among the 7 additional alterations found in a homozygous state, three (S940X, 845delT, dup749-763) lead to a truncated protein while the Q775P mutation affects a SCAP conserved residue in the SSD. All 4 patients had shown a severe infantile neurological disease. A neurological adult-onset patient with mild variant biochemical alterations was found homozygous for a V950M substitution. Among 16 other mutations found in the heterozygous state, M631R and L724P affect the SSD while M1142T and T1205K, located in transmembrane domains, affect residues conserved in human *Patched*. Characterization of the impact at the cellular and biochemical level of mutations with well defined genotype/phenotype correlations should provide clues for better understanding the function of the NPC1 protein.

Program Nr: 2820 from the 1999 ASHG Annual Meeting

In vitro analysis of FTDP-17 missense mutations in 3 and 4 repeat tau in neuronal and non-neuronal stable cell lines. *M.A. van Slegtenhorst, N. Mehta, M. Hutton.* Neurogenetics, Mayo Clinic Jacksonville, Jacksonville, FL.

Mutations in tau have recently been found associated with Fronto-temporal Dementia with Parkinsonism linked to chromosome 17 (FTDP-17). The majority are missense mutations and exon 10 splice-site mutations that effect the ratio of Tau containing 3 and 4 microtubule binding domains. We have created stable lines in CHO and human neuroblastoma M17 cells overexpressing mutant and wild type Tau constructs. They express Tau with 3 or 4 microtubule binding domain repeats and a series of missense mutations (V337M, R406W and P301L).

Western blot analysis shows high expression levels in all lines tested. The CHO lines have been tested for the distribution of mitochondria and preliminary data suggest that there is clustering in all lines overexpressing tau. No qualitative effect on mitochondrial transport could be detected for the different mutations or between Tau with 3 or 4 microtubule binding domains. We are in the process of analyzing the stable lines for differences in free versus bound Tau and the relative cellular levels of Glu-tubulin and Tyr-tubulin.

In addition we have generated GFP-tau in lines with an inducible promotor (Tet-on) to study the effect of the different mutations on microtubule dynamics.

This study was supported by the Kendall Fellowship, Mayo Foundation.

Program Nr: 2821 from the 1999 ASHG Annual Meeting

Incidence of the major Nijmegen breakage syndrome (NBS) mutation 657del5 in Czech Republic, Poland and Ukraine. *R. Varon[1], E. Seemanova[2], K. Chrzanowska[3], D. Abramczuk[3], O. Hnateyko[4], K. Sperling[1], A. Reis[1,5]*. 1) Institute of Human Genetics, Humboldt University, Charite, Berlin, Germany; 2) Department of Medical Genetics, Charles University, Prague, Czech Republic; 3) Department of Medical Genetics, Children's Memorial Health Institute, Warsaw, Poland; 4) Scientific-Research Institute of Hereditory Pathology, Lvov, Ukraine; 5) Mikrosatellitenzentrum, Max-Delbrck Center, Berlin, Germany.

Nijmegen breakage syndrome (NBS) is an autosomal recessive chromosomal instability syndrome, characterised by microcephaly, bird-like face, growth retardation, radiosensitivity, immunodeficiency and high susceptibility to lymphoid malignancy. Most patients are of Slavic ancestry. Recently we cloned the NBS1 gene which codes for nibrin, a member of the RAD50 protein complex of DNA double-strand break repair and recombination. We identified a total of 7 truncating mutations in different NBS patients, the majority being homozygous for mutation 657del5 in exon 6. We found this mutation in 68 unrelated NBS patients. Based on epidemiological data it has been proposed that NBS heterozygotes also have an elevated incidence of malignancies. In order to determine the population incidence of NBS mutations we now undertook a study to estimate the frequency of the 657del5 mutation in the Czech Republic and Poland. We analysed 1713 randomly selected, anonymous Guthrie cards from new-borns, 645 from Czech Republic 568 from Poland and 500 from Ukraine. Using PCR-SSCP analysis followed by direct sequencing of positive samples we identified a total of 11 heterozygotes (5, 3 and 3, respectively), corresponding to an incidence of approx.1 in 156 in the three populations tested. If the elevated cancer risk for NBS heterozygotes is confirmed this high carrier frequency would mean, that this NBS mutation contributes considerably to the cancer load of both populations investigated. Other non-founder mutations in NBS1 could also contribute to the cancer frequency in other populations and confirm the hypothesis that nibrin acts as a tumour-suppressor. We now plan to asses the exact cancer risk for these individuals trough epidemiological studies.

Program Nr: 2822 from the 1999 ASHG Annual Meeting

The human LDL receptor gene (LDLR) database: Molecular analysis of 535 mutations. *M. Varret, J-P. Rabès, L. Villéger, C. Junien, C. Béroud, C. Boileau.* INSERM U383, Hopital Necker, 75015 Paris, France.

To date, 620 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 systematically explored. Thus, we have compiled a database containing standardized data for each LDLR gene mutation, and developed the software that provides sorting tools and allows optimized multicriteria research [http://www.umd.necker.fr]. The analysis of the 535 point mutations in our LDLR database gives the following information: [1] 61% of the mutations are missense, and 20% occur in CpG dinucleotides known to be mutational hot spots; [2] although widely distributed throughout the gene, there is an excess of mutations in exon 4 ($p<0.001$) encoding the 3 central ligand-binding repeats, exon 6 ($p<0.02$) encoding the last ligand-binding repeat, exon 7 ($p<0.05$) encoding the first EGF-like repeat, and exon 9 ($p<0.02$) encoding the NH2-end of the EGF-precursor-like domain; [3] there is a deficit of mutations in exon 13 ($p<0.01$) encoding the COOH-end of the EGF-precursor-like domain, exon 15 ($p<0.001$) encoding the O-linked-sugar domain, exon 16 ($p<0.01$) encoding the transmembrane domain, and exons 17-18 ($p<0.05$) encoding the cytoplasmic domain; [4] 48% of the small deletions occur between repeated sequences and can be explained by the slipped-mispairing model described by Krawczak et Cooper (Hum Genet 1991, 86:425); [5] 69% of the mutations in the ligand-binding domain affect conserved amino-acids for calcium cage formation involved in LDL binding; [6] the functional data we have for 210 (40%) mutations indicate that 33% and 29% of them are class 2B mutations (transport defective alleles) and class 1 mutations (null alleles) respectively; [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 for each mutation and should overcome this shortage.

Program Nr: 2823 from the 1999 ASHG Annual Meeting

Further evidence for a chromosome 4p haplotype segregating with Parkinson's disease in an independent Italian kindred. *J.R. Vaughan[1], M. Farrer[2], G. De Michele[3], G. Volpe[3], J. Hardy[2], N.W. Wood[1]*. 1) University Department of Clinical Neurology, Institute of Neurology, Queen Square, London, WC1N 3BG, UK; 2) Mayo Clinic Jacksonville, Neurogenetics, 4500 San Pablo Road, Jacksonville, Florida 32224; 3) Dipartimento di Scienze Neurologiche, Universita Federico 11, Naples, Italy.

A chromosome 4p haplotype segregating with Parkinson's disease and postural tremor has been suggested as a new locus for Lewy body parkinsonism (4p15). The two branches of the kindred (Spellman-Muenter /Waters and Miller (Iowa kindred)) shared a haplotype spanning the interval from D4S1551 to D4S3350, a genetic distance of 10.7cM. The disorder is characterised by young age at onset, rapidly progressive dopa-responsive parkinsonism and dementia. We report an Italian family where the 4 affected individuals in 2 successive generations all share part of this haplotype for four adjacent markers, D4S2305, D4S2397, D4S1609 and D4S230 (same allele sizes in both families). They have a young onset age and moderately progressive dopa-responsive parkinsonism. Cognitive impairment was present in 2 out of the 4 affected individuals.One individual sharing the haplotype is not yet affected (aged 71) and is not said to have a postural tremor.Three recombinant haplotypes in the Italian family were observed. Assuming intermarker linkage equilibrium, the region of shared haplotype between the Iowa and Italian families spans 5.36cM and has a frequency of 1/374, but due to its small size the pedigree can generate only 1/3.5 odds for linkage. These data are supportive of evidence for linkage and may point to the same gene being involved in the development of the parkinsonian syndrome in both families, but more affected individuals will be needed or a common ancestor demonstrated between the two kindreds if this family is to be used conclusively to study 4p15. Several genes map to 4p15 including ubiquitin C-terminal hydrolase isozyme L1 (PGP9.5)which was negative in the Italian family. Positional cloning strategies will be used to find the causative gene at this locus but this kindred may represent an important link in narrowing down the 4p candidate region.

Program Nr: 2824 from the 1999 ASHG Annual Meeting

Characterization of the mouse Wolf Hirschhorn Syndrome Candidate gene 1. *A.J.M.H. Verkerk[1], J. Schimenti[2], D. Naf[2], E.W. Gutteling[1], R. Florijn[1], G.J.B. van Ommen[1], J.T. den Dunnen[1].* 1) Dept Human and Clinical Genetics, Leiden Univ Medical Ctr, Leiden, Netherlands; 2) The Jackson Laboratory, Bar Harbor, Maine, USA.

The Wolf Hirschhorn syndrome (WHS) is a malformation syndrome associated with a hemizygous deletion of the distal short arm of chromosome 4 (4p16.3). Patients have specific facial characteristics, midline defects and are mentally retarded. Recently a candidate gene, WHSC1 (Wolf Hirschhorn Syndrome Candidate gene 1) has been identified which maps to the 165 kb critical region deleted in patients. The putative 152 kDa protein contains: two PWWP domains, an HMG box, a SET domain and a PHD-type zinc finger, pointing to a function in transcriptional regulation during development. The gene shows a complex expression involving differential splicing and polyadenylation in many embryonic and adult tissues. All WHS patients characterized to date have different deletions which extend far beyond the WHSC1 gene. To facilitate a functional analysis of the gene we are therefore generating mice with a deleted copy of the gene (knock-out) using loxP-technology, such that it facilitates the subsequent construction of more complex genetic rearrangements, e.g. a PWWP-deleted gene to study this domain, which is present in several other proteins. We are currently also characterizing ES-cell lines carrying X-ray induced deletions originating in the mouse Hd gene near the whsc1 locus. Several lines of mice bearing deletions that span the Hd-/Whsc1- interval have been generated and studied and exhibit strain-dependent heterozygous phenotypes consistent with WHS. These include growth retardation, eye colobomata, midline closure defects, asymmetric skulls, seizures, and embryonic/perinatal lethality. We have constructed a PAC map of the murine Whsc1-Hd locus using known genes and markers and use these PACs to analyze the deletions by metaphase-, interphase- and fiberFISH.

Program Nr: 2825 from the 1999 ASHG Annual Meeting

Evidence that revertant mosaicism in a Fanconi anemia group A patient is caused by mitotic gene conversion.

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Fanconi anemia (FA) is an autosomal recessive disorder characterized by cellular hypersensivity to DNA crosslinking agents. While most FA patients are uniformly sensitive to crosslinkers, approximately one third of FA patients are somatic mosaics in regard to diepoxybutane (DEB) sensitivity of lymphocytes. We previously established that this mosaicism is not limited to lymphocytes by demonstrating the loss of the maternally inherited mutation (2815-2816ins19, exon 29) in individually plucked hematopoietic progenitors from a mildly affected 16 year old FA-A patient (IFAR #557/2). The patient is a compound heterozygote; the paternal mutation is a genomic deletion 3' to exon 34. The fibroblasts retained the 2815-2816ins19 mutation in genomic DNA, while the LCL had no evidence of this mutation. In order to demonstrate that loss of the mutation resulted in expression of a normal transcript, we examined cDNA prepared from fibroblasts and from the LCL. PCR amplification of a 1 kb fragment spanning the maternal allele (exons 23-30) generated two bands from fibroblast cDNA: a normal size fragment and a fragment with a 620 bp deletion involving exons 25-29; the breakpoint in exon 29 was 3' to the 2815-2816ins19 mutation. The LCL cDNA produced a single normal band, with no evidence for the maternal mutation. Examination of polymorphisms in genomic DNA from mother, father, and patient, and cDNA from both fibroblasts and revertant LCL from patient, establish that transcripts are produced from both *FANCA* alleles in the LCL. Heterozygosity for polymorphisms in exon 13, 14, 16 and 22 demonstrate that the loss of the maternal mutant allele is not simply a deletion of that allele, but reflects a reversion of the mutation. Loss of heterozygosity (LOH) in the patient for polymorphisms on the revertant maternal allele in exons 30 and 32 is evidence that this reverse mutation was the result of the nonreciprocal transfer of part of the paternal allele for the maternal, by mitotic gene conversion. Despite this natural gene therapy, the patients continues to exhibit progressive marrow failure.

Program Nr: 2826 from the 1999 ASHG Annual Meeting

Molecular genetic analysis of the glypican gene cluster on chromosome Xq26. *M. Veugelers, G. David.* Laboratory for Glycobiology and Developmental Genetics, Center for Human Genetics, Flemish Interuniversitary Institute for Biotechnology (VIB4), K.U.Leuven, Belgium.

The glypicans compose a family of glycosylphosphatidylinositol(GPI)-anchored heparan sulfate proteoglycans. Mutations in *dally*, a gene encoding a *Drosophila* glypican, disturb the pattern of cell division in the nervous system, while mutations in *GPC3*, the gene for human glypican-3, are associated with the Simpson-Golabi-Behmel overgrowth syndrome (SGBS). We have previously shown that some SGBS-patients also have deletions in *GPC4*, another glypican gene which lies adjacent to *GPC3* on Xq26. We have analysed by SSCP and DNA-sequencing the mutation status of *GPC3* and *GPC4* in a set of 17 SGBS-patients, 9 BWS-patients and 3 patients with other overgrowth syndromes (Perlman syndrome and other syndromes). So far, we have identified one patient with a deletion of the last two exons of *GPC3* and the entire *GPC4* gene. Two other patients with deletions of *GPC3* exons could be identified. Five patients showed point mutations in *GPC3*: different types of mutations were observed: frameshift-, nonsense-, missense- and splice-mutations. All identified mutations are predicted to lead to a complete loss of function of the glypican-3 protein. No mutations could be identified in non-SGBS patients. We have also identified three single nucleotide polymorphism (SNPs) in the *GPC4* gene, but except for the total gene deletion in one patient no evidence was found for the presence of pathological mutations in *GPC4* associated with SGBS. Our data indicate that point mutations in *GPC3* are sufficient to cause the Simpson-Golabi-Behmel syndrome.

Program Nr: 2827 from the 1999 ASHG Annual Meeting

RNA Profiling of Global Expression Changes in Progressive Myoclonus Epilepsy (EPM1). *K.I. Virtaneva[1,2], J. Palatini[1], A-E. Lehesjoki[2], A. de la Chapelle[1,2], R. Krahe[1,2].* 1) Div. Human Cancer Genetics, Comp. Cancer Ctr., Ohio State Univ., Columbus, Ohio; 2) Dept. of Molecular Genetics, Folkhälsan Inst. of Genetics, Univ. of Helsinki, Finland.

Progressive myoclonus epilepsy of Unverricht-Lundborg type (EPM1; OMIM 254800) is an autosomal recessive disorder characterized by stimulus-sensitive myoclonus, tonic-clonic seizures and a progressive course with a mean age of onset of 10 years. By positional cloning, cystatin B (*CSTB*) encoding a cysteine protease inhibitor was shown to underlie EPM1. In the majority of EPM1 patients a 12-mer minisatellite repeat expansion in the *CSTB* promoter was identified as the disease-causing mutation. Expansion of the GC-rich repeat disrupts the function of the *CSTB* promoter and causes transcriptional down-regulation, indicating CSTB loss-of-function as the molecular defect in EPM1. The pathophysiological mechanisms underlying EPM1 are currently unclear. We have performed global RNA profiling of expression changes in lymphoblastoid cell lines of two EPM1 patients and one EPM1 carrier with oligo-based GeneChip microarrays (Affymetrix) to simultaneously quantify 6,800 unique human genes. In the EPM1 patients homozygous for the expansion, CSTB mRNA levels were decreased 4.5-fold and 6.6-fold, respectively. The carrier father showed a decrease of -1.5-fold as predicted for a heterozygous individual. Interestingly, in the profiles of both patients CSTB showed the second most decreased transcript level. Thus, we suggest that CSTB could have been identified as the EPM1 gene by functional positional cloning using DNA microarrays. Of 6,800 genes assayed 1,300 genes were expressed. Thirty-six genes were concordantly dysregulated in both EPM1 patients. Of these 36 genes, 17 showed decreased and 19 increased expression. Several of the down-regulated genes are involved in signal transduction and neural biology. Moreover, one of the down-regulated genes constitutes a functional candidate for EPM1 pathogenesis.

Program Nr: 2828 from the 1999 ASHG Annual Meeting

Variant alleles in the CPT2 gene are associated with increased susceptibility to carnitine palmitoyltransferase II deficiency. *G.D. Vladutiu, D. Smail.* Pediatrics, State Univ.of NY at Buffalo,NY.

Adult-onset carnitine palmitoyltransferase II (CPT II) deficiency is a common lipid myopathy characterized by exercise intolerance and myoglobinuria. CPT II deficiency may be even more common due to increasing biochemical and molecular evidence for the existence of manifesting carriers for certain of the disease-causing mutations. The mutation status of patients can be predicted using the ratio of muscle CPT activity to citrate synthase (CPT:CS) as follows: 2 mutations (0.47-1.00), 1 mutation (1.71-2.69), no mutations (4.90-5.79). An increasing number of symptomatic individuals exist who have modest reductions in CPT enzyme activity ("gray zone" patients; CPT:CS = 2.70-3.00) and who are negative for 15 known mutations in the CPT2 gene. As a potential explanation for these partial reductions, we hypothesize that the known CPT2 gene polymorphisms, V368I and M647V, may play a causative role either independently or in association with pathogenic mutations. Using multiplex allele-specific amplification of genomic DNA, we examined the distribution of the polymorphisms in the general population and in suspected heterozygotes and "gray zone" patients. The frequencies of 368I and 647V were 0.43 and 0.11, respectively, in 50 control individuals. Both the I and V alleles occurred more frequently in 14 suspected heterozygotes (368I, 0.75 [p=0.003]; 647V, 0.43 [p=<0.0001]) and in 6 "gray zone" individuals (368I, 0.83 [p=0.02]; 647V, 0.58 [p=<0.0001]). Eight patients suspected of having 2 disease-causing mutations in CPT2 (CPT:CS <1.00) had the general population frequencies of the variants. Homozygosity for the and 368I and 647V alleles in the "gray zone" group was 2.4- and 9-fold higher, respectively, than in the general population. The IV haplotype has been expressed in vitro and shown to augment CPT enzyme deficiency when associated with certain mutations in the gene (Verderio et al. 1995). Variant haplotypes in different combinations may alter the tetrameric configuration of the enzyme protein thus altering its activity. The identification of detrimental haplotypes may make risk assessment feasible. In vitro expression studies are underway. Supported by the MDA.

Program Nr: 2829 from the 1999 ASHG Annual Meeting

Genetic analysis in Spanish cases of dominant cerebellar ataxia. *V. Volpini[1], J. Corral[1], I. Banchs[1], M. Pujana[1], M. Gratacos[1], X. Estivill[1], O. Combarros[2], D. Genis[3], J. Berciano[2].* 1) Molecular Genetics Department, Cancer Research Institute (IRO), Barcelona, Spain; 2) Neurology Service, Hosp Valdecillas, Santander, Spain; 3) Neurology Unit, Hosp J Trueta, Girona, Spain.

Autosomal dominant cerebellar ataxias (ADCA) are a clinically heterogeneous group of neurodegenerative disorders caused by unstable trinucleotide repeat expansions. Five spinocerebellar ataxia genes: SCA1, SCA2, SCA3, SCA6, SCA7 and the gene for DRPLA (dentato-rubro-palido-Luisian atrophy) have been cloned with the finding of an expansion of a CAG repeat which encodes a polyglutamine tract. The exception is SCA8, which consists of an exonic but untranslated CTG repeat. We present here the molecular analysis of 92 unrelated familial and 114 sporadic Spanish cases of spinocerebellar ataxia. For ADCA cases 15% were SCA2, 15% SCA3, 6% SCA1, 3% SCA7, 1% SCA6 and 1% DRPLA. About 58% of ADCA cases remained genetically unclassified. The expanded alleles ranged from 41 to 59 for SCA1, 67 to 77 for SCA3, 25 for SCA6, 38 to 113 for SCA7 and 63 for DRPLA. The highest CAG repeat variation in meiotic transmission of expanded alleles was detected in SCA7, being of +67 units in one paternal transmission and giving rise to a 113 CAG repeat allele in a patient who died at 3 years of age. Meiotic transmissions have also shown a tendency to more frequent paternal transmission of expanded alleles in SCA1 and SCA7. All SCA1 and SCA2 expanded alleles analyzed consisted of pure CAG repeats, whereas normal alleles were interrupted by 1-2 CAT trinucleotides in SCA1, except for three alleles of 6, 14 and 21 CAG repeats, and by 1-3 CAA trinucleotides in SCA2. No SCA and DRPLA mutations were detected in the 114 isolated cases of spinocerebellar ataxia. In order to complete the study, we are currently performing the CTG-SCA8 screening in our ataxia sample.

Program Nr: 2830 from the 1999 ASHG Annual Meeting

Clinical and genetic characterization of 9 Japanese patients with X-linked a-**thalassemia/mental retardation (ATR-X) syndrome.** *T. Wada[1], T. Kubota[2], Y. Fukushima[2], S. Saitoh[1]*. 1) Dept. of Pediatrics, Hokkaido Univ., Sapporo, Japan; 2) Dept. of Hygiene and Medical Genetics, Shinshu Univ., Nagano, Japan.

Mutations of the *ATRX* gene have been reported in patients with the X-linked a-thalassemia/mental retardation (ATR-X) syndrome (MIM 301040). *ATRX* is also involved in Juberg-Marsidi syndrome, Carpenter-Waziri syndrome, and mental retardation syndrome without a-thalassemia. To elucidate further the significance of mutations of *ATRX* in relation to clinical manifestations, we performed clinical and genetic examinations on a series of Japanese patients with ATR-X phenotypes. Nine male patients from 8 families, including one pair of sibs, were included in the study. All patients had typical clinical features for the syndrome with severe mental retardation. HbH inclusions were positive for 7 patients (including sibs), while negative for 2 patients. *ATRX* mutations were screened by RT-PCR followed by direct sequencing, using lymphoblastoid cell lines. Mutations were confirmed using genomic DNA, including that from 3 mothers. We detected 4 missense mutations (N179S, P190L, V1194I, R246C) in the zinc finger domains and 2 missense mutations (V1552C, Y1847C) in the helicase domains. R246C was found in 2 independent patients. No mutation was detected in one patient who was negative for HbH inclusions. Two mothers were carriers for the respective mutation, while one did not have the mutation suggesting a *de novo* origin of the mutation. All mutations except one (R246C) were novel ones. R246C was previously reported in 15 families and these patients demonstrated typical clinical features associated with a wide range of HbH inclusion (0.006-14 %). One patient with R246C was positive for HbH, but another was not, indicating phenotypic variability associated with an identical mutation. Although the presence of HbH or genital abnormalities were not identified constantly, characteristic facies and behavior were extremely similar in these Japanese patients with *ATRX* mutations. Therefore, severe mental retardation associated with characteristic facies and behavior in males, even without presence of HbH or genital abnormalities, should prompt diagnosis of the ATR-X syndrome.

Program Nr: 2831 from the 1999 ASHG Annual Meeting

DHCR7 mutation analysis in Smith-Lemli-Opitz syndrome. *M.R. Wallace[1], R. Trimpert[1], N. Nwokoro[3], P. Arn[2], C. Williams[1], L. Keppen[5], E. Elias[6], D. Abuelo[7], R. Kelley[4].* 1) Pediatrics/Genetics, Univ Florida, Gainesville/Jacksonville, FL; 2) Nemours Children's Clinic, Jacksonville, FL; 3) NIH--NICHD, Bethesda, MD; 4) Kennedy Krieger Institute, Johns Hopkins Uni., Baltimore, MD; 5) Univ South Dakota, Sioux Falls, SD; 6) Harvard Univ, Boston, MA; 7) Brown Univ, Providence, RI.

Smith-Lemli-Opitz syndrome (SLOS, also called RSH syndrome) is an autosomal recessive condition with a frequency in Caucasians of 1/20,000-40,000. It shows variable expressivity, ranging from severe birth defects leading to early death, to mild cases with little more than mental impairment. The underlying defect is a deficit of cholesterol and accumulation of 7-dehydrocholesterol (precursor), due to loss or impairment of the enzyme catalyzing the last step in cholesterol synthesis, 7-dehydrocholesterol reductase (DHCR7). The DHCR7 gene was cloned in 1998, and mutation studies in 19 patients were published. We are surveying for mutations in a panel of 19 additional unrelated patients, by screening for previously-published mutations, sequencing DHCR7 exons, and doing RNA-based analyses. The most common reported mutation, IVS8-1G>C, an intron error causing cryptic out-of-frame splicing, represents 12 of the alleles in our panel. There are three alleles each of the previously-reported T93M and R352W, and 2 patients with R242C and 2 with Y472H (neither previously published). Thus, these five alleles constitute 58% of the mutations. With the exception of the splicing error, all of the mutations to date are missense mutations (many affecting C-terminal amino acids), with no evidence of homozygosity in any patients. We are analyzing parent DNA in many cases, with no evidence for new mutation thus far. PCR-RFLP tests for many of the mutations have been developed, simplifying screening. We also report analysis of an unusual family with multiple affected first cousins, and another family in which there may be two mutations on one allele. Mutation data, cholesterol biochemistry, and clinical severity are being compared (including 5 other affected sibs), and preliminary work suggests we will find a strong correlation between genotype and phenotype.

Program Nr: 2832 from the 1999 ASHG Annual Meeting

Comparison of genotype and glaucoma treament outcome in patients with Axenfeld-Rieger malformations. *M.A. Walter[1], W.G. Pearce[1], E. Héon[2], I.M. MacDonald[1]*. 1) Ophthalmology, Rm. 832, Univ Alberta, Edmonton, AB, Canada; 2) Eye Research Institute of Canada, Univ. Toronto, Toronto, ON, Canada.

Axenfeld-Rieger (AR) malformations are autosomal dominantly inherited developmental disorders in which patients present with developmental anomalies in the formation of the anterior segment of the eye, leading to juvenile-onset glaucoma. Anomalies of the teeth, jaw and umbilicus are often also present. Very recent results have indicated that mutations of either the PITX2 or FKHL7 genes can underlie AR. We have therefore begun to correlate the specific gene mutation with the glaucoma management and outcomes for AR patients. A S82T missense mutation within the FKHL7 forkhead domain was found in the eight affected members of the "RM" family, a Q23X mutation of FKHL7 was found in the nine affected members of the "KA" family, and a R70H mutation of the PITX2 homeodomain was found in the six affected members of the "AD" family. Of these individuals, 5/8 (RM family), 1/9 (KA family), and 3/6 (AD family) developed increased intraocular pressure (IOP) and/or suffered glaucomatous field defects. These very preliminary data are consistent with the previous indication that about 50% of patients with AR malformations develop glaucoma. Importantly, this suggests that patients with mutations in either PITX2 or FKHL7 have a similar propensity to develop glaucoma. However, the increased IOP/glaucoma found in the RM family with the FKHL7 mutation responded well to medical treatment while that in the AD family with the PITX2 mutation was more difficult to treat, with one patient not responding to either medical or surgical treatments. Similar analyses of an expanded AR patient panel are currently underway. Comparisons of the treatment outcomes with the underlying genetic defect in additional AR patients could allow derivation of improved glaucoma treatment courses for AR patients.

Program Nr: 2833 from the 1999 ASHG Annual Meeting

Mutations in the human androgen receptor ligand-binding domain that impair function of the coactivator TIF2, are associated with defective spermatogenesis. *Q. Wang, E.L. Yong.* Obstetrics & Gynecology, National University of Singapore, Singapore.

The effects of androgen are mediated through the androgen receptor (AR), an X-linked member of the steroid/nuclear receptor family of nuclear transcription factors. The action of AR is tripartite, involving the AR, androgens and coactivators such as transcriptional intermediary factor 2 (TIF2). Androgens when bound to the ligand-binding domain (LBD) activate the AR, and TIF2 is necessary for full transcriptional activity. The AR LBD, based on the crystal structures of related steroid receptors, is likely to conform to a canonical fold consisting principally of 12 a-helices (H) in a three-layer sandwich. Mutations of the AR LBD commonly abrogate ligand-binding resulting in complete androgen-insensitivity (AIS), but amino-acid substitutions in the AR LBD have also been identified in patients who present solely with defective spermatogenesis. In our study, several mutations in the AR LBD were found in 5 unrelated patients with impaired spermatogenesis and infertility. They were located in three hot spots in the predicted interhelical regions between H3/H4, H11/H12 and in H7. Unexpectedly, these mutants (N727K, Q798E, M886V) although located in the AR LBD did not affect androgen binding, but yet reduced AR transactivation function by about 30-50% when measured with reporter genes coupled to synthetic, viral (MMTV) or human (PSA) androgen response elements. The transactivation defect of mutant ARs correlated with defective TIF2 function in cotransfection assays. Binding of chimeric mutant AR LBD fragments to the coactivator, TIF2, in the mammalian two-hybrid and GST-pull down assays, were reduced by about 50% compared to the wild type. Thus our LBD mutations reduce AR transactivation function through defective interactions with TIF2. Two AR mutations are located in the predicted interhelical regions H3/H4 (N727K) and H11/ H12 (M886V) which correspond to subdomains in other steroid receptors known to interact with coactivators, and may define a new disease paradigm in which defective coactivator function causes male infertility and defective spermatogenesis.

Program Nr: 2834 from the 1999 ASHG Annual Meeting

How do missense mutations cause disease phenotypes? A paradigm for mechanism - and for modulation. *P.J. Waters[1], M.A. Parniak[2], B.R. Akerman[1], C.R. Scriver[1].* 1) De Belle Laboratory, McGill University-Montreal Children's Hospital Research Inst., Montreal, PQ, Canada; 2) Lady Davis Institute, Montreal, PQ, Canada.

Missense mutations account for 48% of all disease-causing alleles (HGMDdb). Since very few are predicted to ablate directly an enzyme's catalytic site, how do most affect activity and cause disease? As an example we have analysed four missense mutations in the phenylalanine hydroxylase gene (*PAH*), each associated with disease (PKU) and each changing an amino acid distant from the active site.

Mechanism of effect: On expression in transfected human embryonal kidney cells, all four mutations caused reduced PAH enzyme activity in cell lysates (F39L 47%; K42I 30%; L48S 9%; I65T 18%). Immunoreactive PAH protein was similarly decreased; suggesting that these mutations act solely by reducing protein biostability. Study in two further expression systems (*E. coli* and *in vitro* transcription-translation) confirmed that these mutations did not affect specific catalytic activity, or kinetic properties, of the isolated PAH enzyme. Rather, they all caused altered folding/assembly of the enzyme and accelerated cellular proteolytic degradation of the resulting aberrant protein, explaining the reduced protein levels seen in the human cell system. These findings join other new work (Bross et al., Hum. Mutat., in press) to implicate such a mechanism as an emerging paradigm; whereby missense mutations in various genes cause disease phenotypes.

Modulation of severity: Subtle changes in our conditions for transfection and expression in human cells affected general growth and metabolic state of the cells; while the rank order of observed severity of the four mutations was unchanged, levels of PAH activity and protein (as % wild-type) were altered 2- to 3-fold by such manipulations. Thus altered cellular handling of mutant proteins, likely involving protease and chaperone systems, can significantly modulate phenotype in cultured cell systems. This points to similar modulation *in vivo* directly causing variability in phenotype for "single-gene diseases" between individuals with different background genotypes and environmental experience.

Program Nr: 2835 from the 1999 ASHG Annual Meeting

Mutation analysis of two candidate genes within the hereditary neuralgic amyotrophy locus on chromosome 17q25. *G.D.J. Watts[1], M.P. Keller[1], T.P Iismaa[2], P.F Chance[1]*. 1) Univ Washington, Seattle, WA 98195; 2) The Garvan Institute of Medical Research, St. Vincents Hospital, 384 Victoria Street, Sydney NSW 2010.

Hereditary neuralgic amyotrophy (HNA) is an autosomal dominant disorder characterized by episodes of brachial plexus neuropathy. Frequently associated with HNA are dysmorphic features, including hypotelorism, long nasal bridge and facial asymmetries. Linkage analysis has defined the HNA locus to lay between the markers D17S1603 and D17S802, corresponding to approximately 4.0cM. In this study we have screened two candidate genes, the human GALR2 galanin receptor and survivin gene both mapping to the HNA region, for mutations in affected individuals. The hGALR2 gene is a receptor sub-type for the neuropeptide galanin. The hGALR2 gene contains two exons, and encodes 387 amino acids. Survivin is encoded for by 142 amino acids and belongs to the inhibitor of apoptosis protein (IAP) family. These proteins suppress apoptotic cell death and are highly conserved between species. Sequence analysis of the hGALR2 exons showed no mutations in 5 unrelated probands. Sequence data from LCL cDNA for the survivin gene identified a polymorphism (A®C) at codon 129 in two non-related, affected and non-affected, individuals. This change leads to the Lys being changed to Gln, but is unlikely to be the mutation associated with HNA, since the mutation is present in a non-affected individual.

Program Nr: 2836 from the 1999 ASHG Annual Meeting

Haploinsufficiency for COL5A1 expression is a common molecular mechanism underlying the classical form of the Ehlers-Danlos Syndrome (EDS). *R.J. Wenstrup[1], J.B. Florer[1], F. Young[2], W.G. Cole[2]*. 1) Human Genetics, Pavillion Bldg, Children's Hosp Research Fndn, Cincinnati, OH; 2) The Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada.

The classical form of EDS (formerly types I and II) is characterized by fragile, hyperextensible skin, widened dermal scars, ligamentous laxity and autosomal dominant inheritance. Linkage of classical EDS to COL5A1 has been reported in several pedigrees; it has been estimated that as many as two-thirds of classical EDS pedigrees have causative mutations in COL5A1 or COL5A2, the genes encoding the proa1(V) and proa2(V) chains of type V collagen respectively. However, published series based on RT-PCR/SSCP screening methods have reported a comparatively low yield of mutations (10-15% of EDS cell strains). Because RT-PCR/SSCP does not detect mutations that diminish COL5A1 gene expression, we screened 53 probands with classical EDS for COL5A1 haploinsufficiency by detection of two genomic restriction length polymorphisms (RFLPs) in cDNA prepared from cultured dermal fibroblasts. For each cell strain, a 660 bp segment of genomic DNA or cDNA from the 3'UTR of COL5A1 was amplified by PCR and digested with BstU1 and DpnI. Thirty-two of 52 (61%) probands were heterozygous for one or both RFLPs; cDNA was available from 27 for RFLP analysis. Eight of 27 (30%) had only one COL5A1 allele represented in cDNA. Ribonuclease protection experiments indicated that all eight cell strains with apparent expression of only one COL5A1 allele had a reduced ratio of proa1(V):proa2(V) mRNAs compared to 12 control cell strains or to 15 EDS cell strains in which haploinsufficiency was excluded by RFLP analysis. Therefore mutations resulting in haploinsufficiency for proa1(V) chains were present in 30 percent of individual with classical EDS, which are detectable by analysis of expressed RFLPs in COL5A1 and quantitation of proa1(V):proa2(V) mRNA ratios by ribonuclease protection. These data also indicate that unmineralized connective tissues are highly sensitive to quantitative reduction of type V collagen expression.

Program Nr: 2837 from the 1999 ASHG Annual Meeting

A family with PROMM unlinked to the DM2 locus on chromosome 3q22. *T. Wieser[1], D. Boensch[2], K. Eger[1], W. Schulte-Mattler[1], S. Zierz[1]*. 1) Neurologische Klinik, Universität Halle, Halle/S, Germany; 2) Klinik für Neurologie, Universität Jena, Jena, Germany.

Proximal myotonic myopathy (PROMM) is a newly described, autosomal dominantly inherited multisystem disorder similar to myotonic dystrophy. PROMM is characterised by weakness of the legs, myotonia, cataracts, and slight elevation of liver enzymes. Since PROMM patients do not harbour the trinucleotide expansion on chromosome 19 causing Myotonic dystrophy (DM), PROMM is a genetically distinct disease. A recently mapped second locus for myotonic dystrophy (DM2) was thought to be an attractive candidate locus for PROMM, and this hypothesis was supported by reports of linkage to this locus in some PROMM families. We present a German pedigree with PROMM, large enough to formally show or exclude linkage to this locus. Nine markers were used spanning the 8 centimorgan interval comprising the DM2 locus. Lod scores below -2 excluded linkage to this locus. The genetics of the multisystemic myotonic dystrophies besides classical DM seem to be complex. Exclusion of the DM2 locus in our family is further evidence of genetic heterogeneity of PROMM and stresses the broad genetic variety in this small group of disorders, for which the acronym DOMMOPS (dominant myotonic myopathies) was recently introduced. The situation seems comparable to the situation in the dominant ataxias where the broad clinical spectrum underlies an even broader genetic variety.

Program Nr: 2838 from the 1999 ASHG Annual Meeting

Achondroplasia/hypochondroplasia intermediate phenotypes are also associated with the N540K substitution in the FGFR3 gene. *W.R. Wilcox[1], V. Fano[2], L. Chertkoff[2], G. Bonaventure[2], C. Barreiro[2], H. Kitoh[3], D. Kim[4], M. Eswara[5], S. Shelley[5], A. Grix[4], R.S. Lachman[1], H. Lejarraga[2].* 1) Div. Medical Genetics, Cedars-Sinai Med. Cntr., Los Angeles, CA, USA; 2) Depts. of Growth and Development and Genetics, Hosp. de Pediatria J.P. Garrahan, Buenos Aires, ARGENTINA; 3) Dept. Genet., Inst. Dev. Res., Aichi Prefect. Colony, JAPAN; 4) Kaiser-Permanente Hosp., Honolulu, HI and Sacramento, CA, USA; 5) Lucille Packard Childrens Hosp., Palo Alto, CA, USA.

Achondroplasia (ACH) is the most common form of short-limbed dwarfism in humans. In 99% of the cases, the disorder results from the same single amino acid substitution (G380R) in the transmembrane domain of the fibroblast growth factor receptor 3 (FGFR3) gene. Hypochondroplasia (HCH) is a condition similar to but milder than ACH, generally presenting after the second year of life. Approximately 2/3 of HCH patients are heterozygous for a N540K substitution in the intracellular domain of FGFR3 while some families are unlinked to FGFR3. We report 6 patients presenting between 22 weeks of gestation and 1 year of life with short-limbed dwarfism. The clinical and radiographic features range from typical ACH to a phenotype between ACH and HCH. Their growth has been between the 50^{th} and 95^{th} centile for ACH, and their head circumference between the 25^{th} and 50^{th} centile for ACH. An heterozygous N540K substitution in FGFR3 was identified in each case. We conclude that the spectrum of phenotypes for the G380R and N540K substitutions overlap, but the N540K substitution may imply a better prognosis.

Program Nr: 2839 from the 1999 ASHG Annual Meeting

Mutations in Ribosomal Protein S19 gene account for Diamond Blackfan anemia phenotype in 58 out of 216 affected families. *T.N Willig[1], N. Draptchinskaia[2], I. Dianzani[3], S. Ball[4], C. Niemeyer[5], U. Ramenghi[3], K. Orfali[4], P. Gustavsson[2], E. Garelli[3], A. Brusco[3], C. Tiemann[5], N. Dahl[2], N. Mohandas[1], G. Tchernia[1]*. 1) Lawrence Berkeley Laboratory, Berkeley, CA; 2) Unit of Clin. Genet., Dept. of Genet. and Pathol., Uppsala Univ. Child. Hosp., Uppsala, Sweden; 3) Dept of Ped. and Genet., Univ. of Torino, Torino, Italy; 4) DBA Study group, Div. of Haematol., Dept. of Cellular and Molecular Sci., St George's Hosp. Med. School, London, UK; 5) Universität-Kinderklinik, Freiburg, Germany.

Diamond Blackfan anemia (DBA) (OMIM: 205900) has recently been shown to be the first human disease caused by mutations in a gene encoding for a ribosomal protein (RPS19). In order to determine the prevalence of mutations in this gene in DBA, and to describe the molecular basis for the variable clinical phenotype, the genomic sequence of the six exons and the 5untranslated region of the RPS19 gene was directly assessed in DBA index cases from 216 families. Mutations affecting the coding sequence of RPS19 or splice sites were found in 48 cases (22.2 %), while mutations in non-coding regions were found in 10 patients (4.6%). Mutations included nonsense, missense, splice sites defects and frameshift mutations, as well as complete loss of a normal allele and disruption of the gene by chromosomal translocations. A hot spot for missense mutations was identified between codons 52 and 62 of the RPS19 gene, in a new sequence consensus motif W-[YFW]-[YF]-x-R-[AT]-A-[SA]-x-[AL]-R-[HRK]-[ILV]-Y, derived from the alignment of RPS19 amino-acid sequence of 22 species. No correlation between the nature of mutations and the different patterns of clinical expression, including age at presentation, presence of malformations and therapeutic outcome could be documented. Moreover, RPS19 mutations were also found in some first-degree relatives presenting only with isolated macrocytosis and/or high erythrocyte adenosine deaminase activity, but without documented erythroblastopenia. Our findings suggest that other genetic factors may modulate the expression of this gene to account for the variable phenotypic expression, and that haploinsufficiency is likely to play a key role in the pathophysiology of DBA.

Program Nr: 2840 from the 1999 ASHG Annual Meeting

CNGA3 mutations in cone photoreceptor disorders. *B. Wissinger[1], D. Gamer[1], H. Jaegle[1], S. Mayer[1], S. Andreasson[2], T. Rosenberg[2], B. Jurklies[3], E.C. Sener[4], S. Tatlipinar[4], N. Akarsu[4], D.B. Hanna[5], S.G. Jacobson[5], G. Rudolph[3], U. Kellner[3], E. Apfelstedt-Sylla[1], L.T. Sharpe[1], S. Kohl[1]*. 1) University Eye Hospital, Tuebingen, Germany; 2) University Eye Hospital, Lund, Sweden, National Eye Clinic, Kopenhagen, Denmark; 3) University Eye Hospitals Essen, Muenchen and Berlin, Germany; 4) Hacettepe University, Ankara, Turkey; 5) Scheie Eye Institute, Philadelphia, USA.

We have recently shown that mutations in the CNGA3 gene, encoding the cone cGMP gated channel cause rod monochromacy (RM), an autosomal recessive disorder characterized by the absence of color discrimination, photophobia and low visual acuity (Kohl et al., Nature Genet.19: 257-259). Here we present the results of a screening for CNGA3 mutations in patients with RM and other types of cone photoreceptor dystrophies, which enables us to establish a first comprehensive mutation spectrum of the CNGA3 gene. In total 27 missense mutations, 4 nonsense (stop and frameshift) mutations and 1 in-frame deletion, all of them excluded from 100 controls (= 200 chromosomes), were identified. Both mutant alleles could be identified in 28 families (= 56 mutant chromosomes) including 8 cases harbouring homozygous mutations and 20 cases showing compound heterozygosity. In 6 cases only a single heterozygous mutation could be found. 10 of the mutations were observed recurrently in different families. Recurrent mutations mostly share common haplotypes and one of them, Arg283Trp, has been exclusively found in Northern Europe. Mutations were only found in three of the seven coding exons of the CNGA3 gene and cluster in the S4 domain (5 mutations), the cGMP binding domain (9 mutations) and its preceeding linker region (8 mutations). At least in 5 patients with CNGA3 mutations residual cone photoreceptor function associated with better visual function and clear color discrimination abilities was found, indicating that mutations in the CNGA3 gene do not necessarily cause RM.
Supported by the DFG.

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Program Nr: 2841 from the 1999 ASHG Annual Meeting

Placental tissue supporting females with X-linked incontinentia pigmenti does not show skewed X-inactivation.

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Familial incontinentia pigmenti (IP) is an X-linked dominant genodermatosis characterised by male lethality and abnormalities of the skin, hair, teeth, eyes nails and central nervous sytem in affected females. The disorder has been genetically linked to Xq28, a region containing many candidate genes. Examination of candidates is hampered by the fact that affected females exhibit severely skewed X-inactivation of blood and skin cells with early elimination of cells expressing the mutated X. In order to identify a tissue expressing the IP chromosome we have examined X-inactivation patterns in matched samples of cord blood and placenta from 2 affected IP cases using the human androgen receptor gene assay (HUMARA). Both cases were born of an affected mother with skewed X-inactivation and both went on to develop typical IP signs as neonates. Term placental tissue from both subjects showed random X-inactivation whereas cord blood exhibited partial (case 1) or complete (case 2) skewing. Thus, selection against peripheral blood cells in IP occurs at or before term and even cord blood is not a suitable source of RNA for gene analysis. That both X chromosomes were active in placental tissue was confirmed by RT-PCR analysis of X-linked, expressed polymorphisms. These results indicate either that the IP gene is not expressed in placenta or that the IP mutation is not detrimental in this context. Thus we have identified a tissue, placenta, that can be used for screening of candidate genes through direct sequencing of cDNA. We have already used this approach to examine the gene for MTCP-1, an Xq28 locus involved in the control of cell proliferation.

Program Nr: 2842 from the 1999 ASHG Annual Meeting

Differential toxic effects of transition metal ions in Friedreich's ataxia cells. *A. Wong[1], S. Danielson[1], J. Yang[1], F. Taroni[2,3], G. Cortopassi[1]*. 1) VM Molecular Biosciences, UC Davis, Davis, CA; 2) Laboratory of Cellular Pathology, Istituto Nazionale Neurologico "Carlo Besta", Milan, Italy; 3) Biochemistry and Genetics, Istituto Nazionale Neurologico "Carlo Besta", Milan, Italy.

Friedreich's ataxia (FRDA) is an autosomal recessive, neurodegenerative disease characterized by progressive gait and limb ataxia and cardiomyopathy. Expansions of an intronic GAA repeat inhibits transcription of a mitochondrial protein, frataxin. In studies using yeast lacking the frataxin homolog, there is an increase in mitochondrial iron concentration, respiration deficiency, and sensitivity to oxidative stress. These data support the hypothesis that FRDA is a disease of mitochondrial oxidative stress. We have now compared the sensitivity of FRDA and control lymphoblast cell lines to transition metal ions. Both control and FRDA cells exhibited equal sensitivity to ferrous ion, given as FeSO4. However, FRDA cells were more sensitive to ferric ion given as FeCl3 than control cells. Additionally, FRDA cells were more sensitive to Mn2+ compared to control cells. Neither FRDA nor control cells were sensitive to Co2+ (given as CoCl2) and Zn2+ (given as ZnCl2) up to 10 mM. FRDA and controls cells were equally sensitive to Cu2+. In summary we have observed that FRDA lymphoblasts are differentially sensitive to some forms of iron and to manganese, but not to other metal ions. This differential sensitivity may relate to the pathophysiological mechanism of FRDA.

Program Nr: 2843 from the 1999 ASHG Annual Meeting

Autosomal dominant cerebellar ataxia type III: no evidence for a large pathological CAG repeat expansion in the SCA 11 gene. *P.F. Worth[1], E. Dunne[2], A.H. Nemeth[2], N.W. Wood[1]*. 1) Department of Clinical Neurology, Institute of Neurology, Queen Square, London, WC1N 3BG, UK; 2) Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Oxford, OX3 7BN, UK.

Autosomal dominant cerebellar ataxia type III is a relatively pure cerebellar syndrome, with no other major neurological signs. Moderate expansions in the CAG trinucleotide repeat sequence of the SCA 6 gene have been shown to account for around 50% of ADCA III families in our series. Two other loci, SCA 5 and SCA 10, have been identified by linkage analysis of separate ADCA III families. We recently reported linkage of the disease phenotype in a large British family with ADCA III to a fourth locus on chromosome 15q14-21.3 (SCA11). In common with SCA 6 expansion-positive families, this family displays a relatively benign, slowly progressive phenotype, and anticipation has not been observed. In order to investigate the possibility that a pathological expanded CAG trinucleotide repeat is the cause of the disease in this family, we performed the repeat expansion detection method (RED) using a CTG(10) oligonucleotide, on DNA samples from individuals in this family. The largest RED product obtained was 150bp (equivalent to 50 CAG repeats), but this was obtained in all of 10 affected and 7 unaffected members. It has previously been shown that polymorphic sequences on chromosomes 17 and 18 may contain large, presumably non-pathological, CAG repeats in normal individuals. These repeats may therefore confound RED analysis. Using PCR with specific primers to amplify these sequences, we have shown that these loci are not responsible for the 150bp RED products in this family. However, a pathological CAG repeat expansion of 50 or less CAG repeats, analagous to that of SCA 6, remains possible as the mutational mechanism in the SCA 11 gene in this family.

Program Nr: 2844 from the 1999 ASHG Annual Meeting

Connexin 26 (GJB2) gene mutation analysis for hereditary non-syndromic sensorineural deafness. *B.L. Wu[1], V. Lip[1], D. Wattana[1], G.F. Cox[1], M. Kenna[2], B.R. Korf[1]*. 1) Div Genetics; 2) Div ORL, Children's Hospital and Harvard Medical School, Boston, MA.

We describe a simple and sensitive DNA test to detect all mutations in the coding region of the connexin 26 gene, which can be used cost-effectively in clinical-based molecular diagnosis. This three-step sequencing-based assay involves: 1) single tube PCR amplification of the entire coding sequence of the gene from genomic DNA; 2) direct sequencing of the PCR product using two sets of overlapping primers, which will identify most mutations; and 3) direct sequencing of a nested PCR product to identify compound heterozygosity for two different deletions, which otherwise may be difficult to detect. Using this approach, we have unambiguously identified compound heterozygosity for 35delG/167delT in three unrelated deaf individuals. Because 35delG is the most common connexin 26 mutation in the general population and 167delT is the most common in the Ashkenazi Jewish population, compound heterozygosity for these mutations is expected to occur relatively frequently. We have found four new mutations, N206S, E114G , R127H and T26T, in individuals with hearing loss and have detected 14 known mutations present in homozygous, heterozygous, and compound heterozygous states in 18 of 52 unrelated families, including prenatal testing.

Program Nr: 2845 from the 1999 ASHG Annual Meeting

Mutation screening of candidate genes in Rett syndrome. *F. Xiang[1,2], S. Buervenich[1,3], P. Nicolao[4], M.E.S. Bailey[5], L. Edstrom[2], M. Anvret[1,2], Z. Zhang[1,2].* 1) Department of Molecular Medicine, Karolinska Hospital, Stockholm, Sweden; 2) Department of Clinical Neuroscience, Karolinska Institute/Hospital, Stockholm, Sweden; 3) Department of Neuroscience, Karolinska Institute, Stockholm, Sweden; 4) Department of Neurology and Psychiatry (second Neurological Clinic), University of Padova, Italy; 5) Division of Molecular Genetics, Institute of Biomedical and Life Science, University of Glasgow, Glasgow, U.K.

Rett syndrome (RTT) is a neurodevelopmental disorder which appears to affect females only. Its biological and genetic foundations remain unclear. The small numbers of familial cases and available pedigrees have hindered the use of linkage analysis. In this study, we have evaluated six candidate genes for Rett syndrome selected on clinical, pathological and genetic grounds:*UBE1* (Human ubiquitin-activating enzyme E1), *UBE2I* (ubiquitin conjugating enzyme E2I, homologous to yeast UBC9), *GdX* (ubiquitin-like protein), *SOX3* (SRY-related HMG-box gene 3), *GABRA3* (g-aminobutyric acid type A receptor a3 subunit) and *CDR2* (cerebellar degeneration-related autoantigen 2). No mutations were detected in the coding regions of these six genes from ten affected individuals and, therefore, alterations in the amino acid sequences of the encoded proteins can be excluded as having a causative role in Rett syndrome. Furthermore, we investigated the expression of three Rett candidate genes in Xq28 (*GdX, GABRA3* and *L1CAM* [L1 cell adhesion molecule]) by *in situ* hybridization. No gross differences in the mRNA levels of these three genes were observed in neurons of the frontal cortex between normal controls and Rett patients.

Program Nr: 2846 from the 1999 ASHG Annual Meeting

Emerin mutations in Emery-Ddreifuss muscular dystrophy in Israeli families. *Y. Yaron[1], Y. Nevo[2], S. Ahituv[1], C. Legum[1], H. Reznik-Wolf[1], S. Harel[2], R. Shomrat[1], A. Orr-Urtreger[1].* 1) Genetic Inst, Sourasky Medical Ctr, Tel Aviv, Israel; 2) Institute for Child Development and Pediatric Neurology Unit, Dana Children's hospital, Tel Aviv Sourasky Medical Center,Tel Aviv, Israel.

Emery-Dreifuss Muscular Dystrophy (EDMD) is an X linked recessive disorder characterized by progressive muscle wasting , debilitating contractures, and might be life threatening due to cardiomyopathy with arrythmias and atrial paralysis. During the past three years four Israeli families of both Jewish Ashkenazi and non-Ashkenazi origin (10 patients) were referred to our genetic institute with the clinical diagnosis of EDMD for molecular analysis, genetic counseling and pre-natal diagnosis. Direct sequencing analysis was performed in order to detect mutations in the emerin gene which is located on Xq28. Four different mutations were detected: two in exon 2 (333delT and 412 insA) and one in exon 6 (1675-1678delTCCG) that was previously described. All three mutations result in a frame-shift and a stop codon after 30, 4 and 39 amino acids respectively. The fourth mutation was detected in exon-intron 5 splice junction (IVS5+1G>A), causing a splice site mutation. The three novel mutations that were detected reinforce the concept of preponderance of unique mutations in EDMD. Based on our sample and published data, it seems that exon 2 tend to be mutation prone. Two families underwent prenatal diagnosis in four different pregnancies. In two cases, the families chose to terminate the pregnancy of an affected fetus. Direct sequencing is the method of choice for new mutation detection in EDMD and for carrier detection and prenatal diagnosis.

Program Nr: 2847 from the 1999 ASHG Annual Meeting

Generation and analysis of myocilin transgenic mice. *L. Ying[1], J.H. Fingert[3], E.M. Stone[3], V.C. Sheffield[1, 2]*. 1) Howard Hughes Medical Institute, Univ Iowa, Iowa City, IA; 2) Dept Pediatrics, Univ Iowa, Iowa City, IA; 3) Dept Ophthalmology, Univ Iowa, Iowa City, IA.

We have previously determined that mutations in the gene coding for myocilin (MYOC) cause the vast majority of cases of autosomal dominant juvenile-onset primary open angle glaucoma (JOAG), as well as some cases of adult-onset primary open angle glaucoma (POAG) (1). MYOC is expressed in many ocular and non-ocular tissues. The GLN368STOP mutation of MYOC produces a late-onset form of POAG and is the most common known mutation. In order to better understand the pathophysiology of POAG, we developed MYOC transgenic mice containing either the normal human or GLN368STOP variant in an attempt to develop an animal model for primary open angle glaucoma. The normal human MYOC and mutant variant sequences were ligated into the BamHI site of a vector which contains the beta-actin promoter, intron I sequence and a poly-A site. All constructs were sequenced to demonstrate proper orientation and the presence of the desired sequence (normal and mutant). Plasmid DNA was linearized and used to produce transgenic mice on a C57BL/6 x SJL F2 background by DNA microinjection of fertilized embryos. Potential transgenic mice were screened by PCR and southern blot using genomic DNA isolated from tail clippings. A number of founders were identified for both the normal and GLN368STOP mutation. Expression analysis of F1 mice shows that MYOC mRNA is expressed in eyes, brain, heart, kidney, lung, liver, spleen and skeleton muscle. The mRNA transcribed from the GLN368STOP transgene is the same size as the message produced by the normal MYOC gene. Protein analysis using antibodies specific to MYOC and histological evaluation of mature mice are in progress. The expression studies suggest that these transgenic mice will be suitable for further investigation of the role of MYOC in elevating ocular pressure and optic nerve damage.

Stone, E.M. et al (1997) Science 275:668-670.

Program Nr: 2848 from the 1999 ASHG Annual Meeting

Sarcoglycan-sarcospan complex interacts with syntrophins/a**-dystrobrevin as well as the dystroglycan complex.**

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The sarcoglycan (SG) complex composed of four dystrophin-associated proteins (DAPs) termed a- to d-SGs is a subcomplex of dystrophin-DAP complex present on sarcolemma. The SG complex is known to be essential for skeletal muscle survival, since a loss or a remarkable reduction of the SG complex due to a mutation of any one of the SG genes causes a group of diseases termed sarcoglycanopathy, which is a Duchenne-like muscular dystrophy inherited with an autosomal recessive mode. Nevertheless, the higher order structures surrounding SG complex in dystrophin-DAP complex, which are the basis for understanding the function of SG complex, had been ill-defined. In order to understand these structures, we explored the methods to prepare the SG complex holding interactions with remainders of dystrophin-DAP complex and discovered the methods by which three kinds of DAP complexes containing the SG complex are prepared. The complexes are 1) the SG-sarcospan (SPN) complex, 2) the SG-SPN-syntrophins/a-dystrobrevin complex and 3) the SG-SPN-dystroglycan complex. Based on these results, we conclude that the SG complex is associated with SPN and that this SG-SPN complex interacts with both syntrophins/a-dystrobrevin and the dystroglycan complex. Since the presence of SG complex is fundamental in these connections, its loss or great reduction would make serious effects on muscles, which causes sarcoglycanopathy.

Program Nr: 2849 from the 1999 ASHG Annual Meeting

Intrafamilial variability of myocardial hypertrophy and modifying factors in familial hypertrophic cardiomyopathy (FHC). *B. Yu[1,3], R.W. Jeremy[2,3], D.R. Richmond[2,3], R.J. Trent[1,3].* 1) Dept Molecular & Clinical Genetics, Royal Prince Alfred Hosp; 2) Dept Cardiology, Royal Prince Alfred Hosp; 3) Dept of Medicine, University of Sydney, Australia.

FHC is an autosomal dominant disease caused by >120 family-specific mutations in at least 7 genes coding for sarcomere proteins. Clinically it is characterised by left ventricular hypertrophy (LVH). To identify modifying factors, the severity of LVH was analysed in relationship to potential modifiers in 40 mutation carriers from two FHC pedigrees. In family W, eight individuals carried an Arg453Cys mutation in the cardiac b-myosin heavy chain gene. Complete penetrance was observed. The proband had mild LVH and died of heart failure aged 53. Her 9 year-old granddaughter had massive LVH and started to develop cardiac insufficiency aged 12. The proband's daughter with the same mutation was asymptomatic in her 30s with minimal LVH. In four generations of family S there is a Gln966X mutation in the cardiac myosin binding protein-C gene. Only 14 of 32 mutation carriers had cardiac hypertrophy. A range of potential modifying factors were explored. These included: age, gender and several candidate genes. Intrafamilial variations in family W could not be accounted by age, sex and environmental factors such as exercise. However, the severity of LVH in family S was significantly correlated with age ($r=0.76$, $p<0.001$). The expression of LVH in two families was not due to an over-representation of the D allele in the angiotensin-I converting enzyme gene. According to the affected pedigree member analysis, there was no significant association between the myotonin gene and those with clinically detectable LVH in both families. However, significant allele-sharing was detected between the androgen receptor gene and clinically affected mutation carriers in family S ($p=0.003$). This association was supported by multipoint linkage analysis using Genehunter (NPL scores>2.1, $p=0.021$). The association remained significant when the clinically affected individuals in family W were added. Our results indicate that although FHC is a monogenic disease, the development of LVH can be influenced by multiple factors.

Program Nr: 2850 from the 1999 ASHG Annual Meeting

Disruption of mouse kynurenine aminotransferase II gene, a possible factor in the pathophysiology of Huntington's disease. *P. Yu[1], A. Chen[1], R. Schwarcz[2], D. Tagle[1]*. 1) NIH/NHGRI, Bethesda, MD; 2) Maryland Psychiatric Research Center,University of Maryland School of Medicine, Baltimore.

Decreased levels of the endogenous neuroprotectant kynurenic acid (KYNA) have been observed in the brain of Huntington Disease (HD) patients and may contribute to neuronal loss in this disorder. This reduction may be caused by a dysfunction of kynurenine aminotransferase II (*Kat-2*), the major enzyme responsible for the synthesis of KYNA in the brain. Towards understanding the role of *mKat-2* in kynurenine pathway and HD, we disrupted *mKat-2* gene in the mouse. To date, we have obtained a total of 30 *mKat-2*$^{+/+}$, 62 *mKat-2*$^{+/-}$ and 36 *mKat-2*$^{-/-}$ F_2 mice from 6 founder lines with the gender and genotypes reflecting the expected Mendelian frequency. There are no detectable *mKat-2* protein and mRNA in the homozygous knock out mice by Western and Northern blot analysis. Homozygous *mKat-2*$^{-/-}$ mice were phenotypically indistinguishable from their littermates at birth. However, at 40 days of age a noticeable forelimb clasping posture can be elicited in less than a minute upon tail suspension of *mKat-2*$^{-/-}$ mice but not in littermate controls. By 50 days of age, nullizygous mice have progressed to display clasping responses involving all four limb upon tail suspension, similar to the earlier stage phenotype of HD mice model developed in our laboratory (Nature Genet 1998; 20:198-202). The pathological evaluation of brain sections from *mKat-2*$^{-/-}$ mice including hematoxylin and eosin (H & E) stain, glial fibrillary acidic protein (GFAP), and Nissl are currently underway. KYNA-deficient mice would be useful in determining the link between kynurenine pathway metabolism and HD pathogenesis. In addition it may provide relevant information regarding the role of kynurenines in excitatory amino acid (EAA)-related physiology and pathology.

Program Nr: 2851 from the 1999 ASHG Annual Meeting

Haplotype analysis of b-**thalassaemia mutations in Lebanon.** *L.F. Zahed[1], M. Qatanani[1], M. Nabulsi[2].* 1) Dept of Pathology and Laboratory Medicine, American Univ Beirut,Beirut, Lebanon; 2) Dept of Pediatrics, American University of Beirut, Beirut, Lebanon.

Studies on the molecular basis of b-thalassaemia in Lebanon have revealed the presence of 18 different mutations, reflecting the heterogeneity of the lebanese population, which is known to be a mosaic of individuals of different religious and ethnic origins. We have proceeded to study the haplotypes linked to these mutations, by analyzing seven restriction sites in the b-globin gene cluster. A total of 108 chromosomes were analyzed for the following sites: HindIIe, HindIIIGg, HindIIIAg, HindII5'yb, HindII3'yb, AvaIIb and HinfIb. Twelve different haplotypes were identified. While the linkage of many mutations to particular haplotypes was as previously reported for Mediterranean populations, some unusual linkages were observed, such as the linkage of an African haplotype to IVSI-110, the most frequent mutation in the Eastern Mediterranean. This linkage was exclusively found among individuals from a particular community. Many mutations were also found to be linked to different haplotypes: these include both common and rare mutations. Haplotype analysis in conjunction with b-thalassaemia mutations will allow us to retrace the origin of the different groups making up our population.

Program Nr: 2852 from the 1999 ASHG Annual Meeting

Evidence for genetic heterogeneity in Leydig cell hypoplasia. *J.C. Zenteno[1], P. Canto[2], S. Kofman-Alfaro[1], J.P. Mendez[2]*. 1) Genetics, Hospital General Mexico UNAM, Mexico City, Mexico; 2) Research Unit in Developmental Biology, CMN SXXI, IMSS, Mexico City, Mexico.

Leydig cell aplasia or hypoplasia is a rare form of male pseudohermaphroditism resulting from inadequate fetal testicular Leydig cell differentiation. Affected individuals presented a wide phenotypic spectrum ranging from complete female external genitalia to males with micropenis. Recessive mutations in the LH receptor gene have been identified as responsible of the condition. The majority are point mutations and have been located in exon 11 of the gene. We report the molecular characterization of the LH receptor gene in a familial case of Leydig cell hypoplasia where the presence of an already described polymorphism in exon 11 excludes that a mutation in this gene is responsible of the disorder in this particular family. A non-consanguineous Mexican family with 3 affected individuals was studied. The patients presented a female phenotype,primary amenorrhea, absence of secondary sexual characteristics, intraabdominal testes and an 46,XY karyotype. Testicular histology revealed seminiferous tubules lined by Sertoli cells and absence of mature interstitial Leydig cells. Genomic DNA was obtained from peripheral blood leukocytes of the three patients, their father and a healthy sister. PCR amplification and direct automated sequencing of the LH receptor gene were performed in all subjects. After sequencing the 11 exons of the gene, no deleterious mutations were detected in any patient. However, we identified a previously described silent polymorphism in exon 11: in patients 1 and 3 DNA sequencing revealed a C to T substitution at nucleotide 1065, being both patients homozygous GAT/GAT at codon 355. In contrast, patient 2 was homozygous GAC/GAC at this polymorphic site. These results exclude that Leydig cell hypoplasia in this family is due to a mutation in the LH receptor gene and provide evidence that defects in other loci may also result in failure of Leydig cell differentiation demonstrating for the first time, that Leydig cell hypoplasia is a genetically heterogeneous condition.

Program Nr: 2853 from the 1999 ASHG Annual Meeting

Functional Consequences of Chloride Channel Gene (*CLCN1*) Mutations Causing Myotonia Congenita. *J. Zhang[1,2], S. Bendahhou[4], L.J. Ptacek[1,2,3,4].* 1) Neuroscience Program, Univ Utah, Salt Lake City, UT; 2) Department of Neurology, Univ Utah, Salt Lake City, UT; 3) Department of Human Genetics, Univ Utah, Salt Lake City, UT; 4) Howard Hughes Medical Institute, Univ Utah, Salt Lake City, UT.

Myotonia congenita (MC) is a genetic muscle disease with two inherited forms: autosomal dominant (Thomsen's disease) and autosomal recessive (Becker's diseases). MC is associated with membrane hyperexcitability due to abnormalities in the skeletal muscle voltage-gated chloride channels (ClC-1). In skeletal muscle, ClC-1 channels contribute 80% of total resting transmembrane conductance and determine the membrane excitability. Mutations have been identified in ClC-1 gene (*CLCN1*) of MC patients. In order to understand the molecular basis of this inherited disease, and to prove the mutations are disease associated defects, it is important to determine the physiological consequences of uncharacterized mutations found in MC patients. We used a mammalian cell (HEK-293) expression system and whole-cell voltage clamp technique to functionally express and physiologically characterize five *CLCN1* mutations. Both I329T and R338Q mutant channels shifted the voltage dependence of open probability of ClC-1 channels to more positive potentials. In addition, the I329T mutant channels deactivated to a lesser extent than normal at negative potentials. V165G, F167L, and F413C mutant channels also shifted the voltage dependence of open probability, but only by +14 to +20 mV. The functional defects caused by these mutations could result in the reduced macroscopic chloride conductance in muscle membrane, which could impair the ability of the ClC-1 channels to maintain normal muscle excitability and cause myotonia congenita.

Program Nr: 2854 from the 1999 ASHG Annual Meeting

New Mutations in RPGR and RP2 and a New Locus for X-linked Retinitis Pigmentosa. *I. Zito[1], D.L. Thiselton[1], M. Gorin[2], A.C. Bird[1], S.S. Bhattacharya[1], A.J. Hardcastle[1].* 1) Depts. of Molecular Genetics and Clinical Ophthalmology, Institute of Ophthalmology, London, UK; 2) Dept. of Ophthalmology, School of Medicine, University of Pittsburgh, USA.

Purpose. To establish the spectrum of mutations in the RPGR and RP2 genes causing X-linked retinitis pigmentosa (XLRP), and define the number of distinct loci on the X-chromosome involved in retinal disease. **Methods.** A comprehensive screen for RPGR mutations was undertaken for all 19 exons by SSCP analysis, and RP2 mutations were detected by sequencing all 5 exons of this gene. Segregation of mutations with disease was demonstrated by SSCP analysis, sequencing or agarose gel electrophoresis. Haplotype analysis was performed with over 34 polymorphic markers spanning the entire X-chromosome. **Results.** A subset of 30 XLRP families, where disease segregates with the RP3 locus, were selected for RPGR mutation screening. 6 novel mutations in RPGR have been identified and this analysis includes the first reported mutations in exon 1. RPGR mutations therefore account for 20% of disease in families with an RP3 genotype. We also identified 8 families, from a total of 61, with mutations in the RP2 gene accounting for approximately 13% of familial XLRP. We have extensively haplotyped many XLRP families and here we report a new locus for XLRP (RP23) in a single family. Disease maps to a 14 cM region on the distal short arm of the X-chromosome (Xp22) between markers DXS1223 and DXS7161. **Conclusions.** These results describe mutations in 14 of our XLRP families. We have also identified a new locus for XLRP (RP23) adding to the level of heterogeneity for this disease. A large proportion (up to 70%) of XLRP remains unaccounted for by combined genetic mapping and gene mutation studies.

Program Nr: 2855 from the 1999 ASHG Annual Meeting

Screening for mutations of cardiac actin gene in dilated cardiomyopathy. *R. Zolty[1], G.L. Brodsky[1], P.A. Underhill[2], L.L. Cavalli-Sforza[2], M.R. Bristow[1], L. Mestroni[1].* 1) Molecular Genetics, CU-CVI, Aurora, CO; 2) Dept. of Genetics, Stanford University, Stanford,CA.

Idiopathic dilated cardiomyopathy (DC) is frequently inherited and genetically heterogeneous. Recently, mutations in exons 5 (Arg312His) and 6 (Glu361Gly) of the human cardiac actin gene (ACTC) have been found to segregate with the disease in 2 families with DC. In order to evaluate prevalence and characteristics of cardiac actin gene mutations in DC, 65 DC patients have been studied: 17 with sporadic DC and 48 with documented familial DC (belonging to 32 unrelated families); 2 patients with ischemic heart disease were used as controls. Genomic DNA was extracted from blood or explanted heart tissue using standard procedures. PCR products were generated 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. Exon 5 amplimers generated from all patients were digested with Bcl1/Nde1. None of the exon 5 amplimers showed the presence of the expected restriction fragments. Mutation analysis of all 6 exons is currently being performed, using PCR amplification with allele-specific oligonucleotide primers for the A-to-G transversion in codon 361 of exon 6, and DHPLC and sequencing for novel mutations. Our preliminary results indicate that the Arg312His mutation in exon 5 is not responsible for the disease in a large population of familial DC patients and in a pilot group of sporadic DC cases, and is probably rare. The results of the fine mutational analysis of ACTC gene currently in progress will add important information about the relevance of this gene in the pathogenesis of DC.

Program Nr: 2856 from the 1999 ASHG Annual Meeting

Pulmonary hypertension (PHT) in type 1 Gaucher disease : Prevalence and impact of enzyme replacement therapy(ERT). *P.K. Mistry[1,2], H. Chung[1], S. Wallenstein[3], R.J. Desnick[1], M. Goldman[2]*. 1) Departments of Human Genetics,; 2) Medicine,; 3) Biomathematics, Mount Sinai School of Medicine, New York.

In Gaucher disease(GD), deficiency of lysosomal glucocerebrosidase leads to widespread accumulation of macrophages engorged with glucosylceramide-laden lysosomes. Although, the lungs represent a major site for deposition of pathological macrophages, overt pulmonary disease is rare, presenting as infiltrative/fibrotic lung involvement or PHT. Angiotensin converting enzyme (ACE) is secreted abundantly by Gaucher cells and it has been implicated in pathogenesis of primary PHT. We screened 103 patients with type 1 GD (19 untreated and 81 on ERT with Alglucerase and/or Imiglucerase for 6-105 months; age 12-82 yrs; 3 patients were excluded because of other underlying causes for PHT) for presence of PHT using doppler echocardiography to estimate physiologic tricuspid regurgitant gradient(TIG, with pressure >25 mm Hg defined as PHT). The GBA genotype of the patients was N370S/N370S in 42 patients, N370S/84gg in 24 and N370S/L444P in 14. The prevalence of PHT in untreated patients was 32% vs 18% among patients on ERT. On univariate analysis, age was significantly correlated with PHT(P=0.0012). Females were over-represented among patients with PHT(27% vs 12% TIG in females was 19.9 mm Hg vs 17.0 mm Hg, p=0.06). There was no relationship of PHT with GBA genotype, serum ACE levels, ACE polymorphisms or splenectomy status. ERT was associated with slight reduction of TIG (21 vs 16.5 mm Hg, p=0.04). On multivariate analysis, age emerged as the stongest risk factor for PHT in GD. The results indicate a hitherto unrecognized, high prevalence of sub-clinical PHT in type 1 GD, consistent with the lungs being a major site of Gaucher cell infiltration. Older age and female sex emerge as additional risk factors for development of PHT in GD. There is no association between PHT and ACE polymorphisms or serum ACE levels. ERT appears to preclude PHT, but further evaluation is required to assess its effect in reversing this complication compared to visceral and bone marrow disease.

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Program Nr: 2857 from the 1999 ASHG Annual Meeting

The Repopulation Potential of Different Size Fractions of Hepatocytes in the Serial Transplantation Assay. *E. Montini[1], K. Overturf[3], M. Al-Dhalimy[1], M. Finegold[4], M. Grompe[1,2]*. 1) Dept. Mol.& Med. Gen., Oregon Health Sci. Univ., Portland, OR; 2) Dept. of Pediatrics, Oregon Health Sci. Univ., Portland, OR; 3) Wallace Tumor Inst.Comprehensive Cancer Center University of Alabama, Birmingham, AL; 4) Dept. Pathol., Texas Childrens Hospital, Houston, TX.

The mouse deficient for the enzyme fumarylacetoacetate hydrolase (FAH) is a model of the human disease hereditary tyrosinemia type I (HT1) and can be used to study basic aspects of liver repopulation by transplanted cells. Using serial transplantation we have previously shown that adult mouse liver contains cells capable of dividing > 100 times in vivo without loss of function. The question whether all hepatocytes or only a sub-population (stem cells) possessed this regenerative ability remained unanswered. In order to address this problem we used centrifugal elutriation to separate hepatocytes by cell density. Unexpectedly, small diploid hepatocytes (16 m) had lower repopulation capacity during the first round of transplantation when compared to both the medium-sized (21 m) and large cells (27 m). Our initial experiments did not explore the long-term repopulation potential of the different size fractions as determined by serial transplantation. Thus it still was possible that the small, diploid hepatocytes harbored the subpopulation capable of more than 100 doublings. We therefore performed 1:1 competitive repopulation between genetically marked 16 m cells and unfractionated hepatocytes in combination with serial transplantation. Analysis of DNA from livers of recipient repopulated in the second round of serial showed that the contribution from the small cells remained unchanged to round 1. Using a competition assay we also compared the repopulation capacity of hepatocytes that had undergone different degrees of prior *in vivo*expansion. Previous cell division neither reduced nor increased the repopulation capacity of transplanted liver cells. Our data indicate that the serially transplantable liver repopulating cells are the differentiated 21 m hepatocytes themselves.

Program Nr: 2858 from the 1999 ASHG Annual Meeting

***In vivo* modulation of the splicing pattern of CFTR exon 9 by cellular and viral splicing factors.** *M. Nissim-Rafinia[1], O. Chiba-Falek[1], E. Kerem[2], B. Kerem[1].* 1) Department of Genetics, The Hebrew university, Jerusalem, Israel; 2) Department of pediatrics and CF Clinic Shaare Zedek Medical Center Jerusalem Israel.

Disease variability among CF patients carrying the same splicing mutation is associated with variable levels of aberrantly spliced CFTR transcripts, suggesting a variability among individuals in the efficiency of exon recognition by trans-acting factors. Here we studied the effect of overexpression of cellular and viral splicing factors, known to promote exon inclusion or skipping, on the splicing pattern of CFTR exon 9. Transient transfection of minigenes carrying the IVS8-5T (p5T), IVS8-7T (p7T) and IVS8-9T (p9T) alleles, to NIH3T3 and COS-1 cells, resulted in their expression and alternative splicing. RT-PCR analysis showed that the efficiency of exon 9 recognition in the pT(n) minigenes was inversely correlated with the length of the pyrimidine tract, as in individuals carrying these alleles. The level of exon 9 skipped RNA transcribed from p5T in COS-1 and NIH cells was 22%±6% and 34%±6%, respectively. Cotransfection of p5T with E4-ORF3, led to a substantial promotion of exon inclusion, resulting in only 9%±3% skipped RNA. Cotransfection of p7T with E4-ORF3 also promoted exon inclusion. Cotransfection of p5T with E4-ORF6 had no effect. Next we studied the effect of the cellular splicing factors ASF/SF2 and hnRNP A1. Cotransfection of p5T with ASF/SF2 to COS-1 and NIH cells, promoted exon skipping to 36%±5% and 57%±3%, respectively. ASF/SF2 had no effect on p7T or p9T. Cotransfection of p5T with hnRNP A1 also promoted skipping to 44%±11% in COS-1 cells, and had no effect in NIH3T3 cells. Our study suggests that in vivo overexpression of cellular and viral factors can affect the splicing pattern of CFTR exon 9 pre-mRNA. Interestingly, the effect of the studied factors on the level of skipped RNA was higher in NIH3T3 than in COS-1.This might indicate that the mechanism of exon recognition is a complex network of genetic and environmental interactions, which might differ among organisms and in a tissue-specific manner. These results are important for understanding the basis of disease variability and for the development of therapies for patients carrying splicing mutations.

Program Nr: 2859 from the 1999 ASHG Annual Meeting

The use of antisense hammerhead ribozymes in an animal model of osteogenesis imperfecta. *J. Niu[1], M.W. Kilpatrick[1], R.J. Wenstrup[2], P. Tsipouras[1]*. 1) Pediatrics, UConn Health Center, Farmington, CT; 2) Human Genetics, Children's Hospital Research Foundation, Cincinatti, OH.

Nearly all forms of Osteogenesis Imperfecta (OI) are caused by mutations in the two genes for type I collagen (COL1A1 and COL1A2). There is considerable evidence that the more severe OI phenotypes are due to the mutant gene product exerting a dominant negative effect. That is, the phenotypic consequences of heterozygous mutations that alter the structure of type I collagen monomers are usually much greater than those which result from simple functional loss of an allelic product. Antisense hammerhead ribozymes are small catalytic RNA molecules that can be designed to cleave a target RNA molecule that contains a putative NUY cleavage site (where N is any base and Y any base except G). Hammerhead ribozyme technology designed to reduce or eliminate expression of a mutant collagen gene is therefore a potentially useful approach to patients with dominant-negative mutations. Hammerhead ribozymes were designed to specifically target a human COL1A1 minigene which is deleted for exons 6 through 46. Expression of this construct in transgenic mice and in the murine calvarial cell line MC3T3-E1 results in reproducible phenotypic and biochemical abnormalities. The ribozyme COL1A1RZ547, targeted to the junction between COL1A1 exons 5 and 47, specifically cleaved its target, the product of the COL1A1 minigene, in an efficient manner. The ribozyme did not cleave a control RNA which was not deleted for exons 6 through 46. Similarly, a control inactive ribozyme molecule cleaved neither the minigene RNA nor the normal control RNA. The COL1A1RZ547 ribozyme is being delivered to MC3T3-E1 cells that stably express the minigene. Delivery is by receptor-mediated endocytosis via the transferrin receptor. The ribozyme will be tested for cleavage efficacy and reversal of the well defined biochemical phenotype of the cells by northern and western blot analysis, pulse chase labelling of procollagens and measurement of several markers of cellular differentiation. In this way, the potential of this ribozyme to ameliorate phenotypic features of OI will be evaluated.

Program Nr: 2860 from the 1999 ASHG Annual Meeting

Cyclophosphamide Mediated Selection of Hematopoietic Stem Cells Corrected by Gene Therapy in a Model of Fanconi Anemia. *M. Noll[1], R.L. Bateman[1], A. D'Andrea[2], M. Grompe[1]*. 1) Dept. of Molecular & Medical Genetics, Oregon Health Sciences Univ., Portland, OR; 2) Dept. of Ped. Onc., Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA.

Fanconi's Anemia (FA) is an autosomal recessive disorder characterized by birth defects, increased incidence of malignancy and progressive bone marrow failure. Bone marrow transplantation is therapeutic and therefore FA is a candidate disease for hematopoietic gene therapy. The frequent finding of somatic mosaicism in blood of FA patients has raised the question, whether wild-type bone-marrow has a selective growth advantage. We have previously shown that mitomycin C (MMC) can be used to select for wild-type stem cells in transplanted FA group C knockout (FACKO) mice. Like MMC, cyclophosphamide (CPA) is a DNA damaging agent and produces interstrand DNA cross-links. However, in contrast to MMC, CPA is already used in human FA patients for preparation during bone marrow transplantation. Since the dose tolerated by human FA patients is known, CPA could potentially be used for enhancement of human FA gene therapy. Preliminary experiments determined that the LD50 of this compound in FACKO mutant mice was four-fold lower than for wild type mice (~ 140 mg/kg vs. 600 mg/kg). Therefore, FACKO mice were transplanted with 1 x 106 wild-type marrow cells without prior radioablation. Transplanted animals were then given a single dose of either 75-140 mg/kg of CPA. In contrast to untreated mice, CPA-treated mice showed significant percentage of wild-type DNA to peripheral blood. The wild-type contribution ranged from ~ 20% at the lower doses to >80% at the higher doses after 8 weeks. Serial transplantation showed that the selection occurred at the level of hematopoietic stem cells (HSCs). We next used a VSVG pseudotyped MMLV vector expressing the human FANCC cDNA to transduce FACKO mutant marrow cells. Similar to the results of obtained with transplanted wild-type cells, a single dose of CPA produced significant selection of retrovirally corrected HSCs. We conclude that cyclophosphamide could be used to enhance stem cell selection in gene therapy of Fanconi anemia.

Program Nr: 2861 from the 1999 ASHG Annual Meeting

Effects of dextromethorphan in autistic children with abnormal plasma amino acid profiles. J.A. Phillips III, R. Hamid and S. McGrew. Dept. of Pediatrics, Division of Medical Genetics, Vanderbilt University. School of Medicine, Nashville, TN. *J.A. Phillips III, R. Hamid, S. McGrew.* Dept Pediatrics/Genetics, Vanderbilt Univ Sch Medicine, Nashville, TN.

Autism is characterized by deficits in sociability, reciprocal communication, repetitive behavior and onset by 3 years of age. Most (75-80%) autistic individuals have primary autism in which no underlying cause is found. Previous studies have implicated derangements of neurotransmitters such as serotonin, norepinephrine and histamine in some cases of autism. In screening a series of consecutive autistic probands, we detected 5/36 (14%) who had elevated plasma levels of glutamine (Gln) or glycine (Gly) or both on repeated studies. Patient one was diagnosed as having autism at 2 10/12. His Gln (mean 884-normal 370-682) and Gly (mean 379-normal 120-315) levels were consistently elevated. Urine Gln and Gly, levels were 1410 (165-510), 5663 (569-1395) respectively. His CSF Gln was 708 (normal 356-680) while his Gly level was normal. His routine chemistry labs including his urine organic acid profile was normal. Patient two had persistent elevated Gly levels on multiple plasma amino acid profiles. These levels ranged from 324-439 (normal for age 120-315). These patients were empirically treated with dextromethorphan (DM) at 5 mg/kg/day (Delsym) divided BID. DM blocks the NMDA (N-methyl-D-aspartate) type receptor in the brain which has an accessary binding site for Gly. Both patient's special education and classroom teachers and speech and occupational therapists (blinded to treatment) reported significant improvement in their expressive and receptive language skills, attention span and focus; motor planning and socialization with peers. After withdrawal of DM regression in all improved areas was noted by all of these treatment blinded observers. These patients continue to respond to resumed, long term DM treatment. Our data suggest some autistic children have consistent elevations of Gln, Gly or both and they may respond clinically to DM. Further studies are needed to determine the cause of these biochemical findings and to quantitate the response of other subjects to DM or other NMDA inhibitors.

Program Nr: 2862 from the 1999 ASHG Annual Meeting

Adenoviral vectors modified for increased CFTR expression and persistence in human ciliated airway epithelia.

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Cystic fibrosis (CF) is a genetic disease resulting from mutations in the chloride channel Cl^-) encoded by the cystic fibrosis transmembrane conductance regulator (CFTR) gene. The apical membrane of CF airway epithelia lacks functional CFTR, resulting in a loss of cAMP-mediated transepithelial Cl^- transport. Gene therapy for CF would require the delivery of a corrected CFTR gene to the airway in sufficient quantity to result in a measurable therapeutic index. Recombinant adenoviruses (Ad) are being designed as vectors for gene transfer to a wide variety of cells and tissues, including the respiratory epithelium of patients with CF. Currently, Ad-based gene transfer is limited in part by the low efficiency of viral entry into human airway cells. Achieving CFTR expression levels that can impart a corrective therapeutic index may thus rely on maximizing gene expression from DNA templates that gain entry into the cell. We have tested the ability of viral vectors altered in their CFTR cassettes to provide high and sustained levels of gene expression in the lungs of immunocompetent BALB/c mice and, *in vitro*, in ciliated human airway epithelia from CF donors. We show that incorporation of an intron upstream of the CFTR cDNA and an a-globin mRNA stability element (aSE) downstream of the transcription termination site each increased the level of CFTR gene expression, both *in vitro* and *in vivo*. A keratin 18 (K18) gene enhancer/promoter directed functional CFTR expression in human airway epithelia, at levels equal to that of the CMV promoter at day 3. Unlike the CMV promoter, whose activity declined over time, K18 provided functional and stable CFTR expression over the course of 30 days. These results suggest that correction of the CFTR defect in human airways should be attempted by using a tissue-specific enhancer and promoter combined with *cis*-acting elements that provide more efficient mRNA stability and translatability. Enhanced expression and persistence of CFTR may be important factors in allowing lower vector doses and longer intervals between readministration.

Program Nr: 2863 from the 1999 ASHG Annual Meeting

Towards a prevention of cardiomyopathy in Friedreich ataxia by idebenone. *P. Rustin, K. Chantrel-Groussard, J.C. von Kleist-retzow, A. Munnich, A. Rotig.* INSERM U-393, Hopital Necker Enfants Malades, Paris, France.

Friedreich Ataxia (FRDA) is a frequent cause of autosomal recessive spinocerebellar degeneration and hypertrophic cardiomyopathy, ascribed to mutations of the frataxin gene. We have reported a generalized deficiency of mitochondrial iron-sulfur proteins (ISPs) in endomyocardial biopsies of FRDA patients (Nat. Genet. 1997, 17:215-217). Reduced iron triggered similar damages to ISPs in human heart homogenates. Reduced idebenone efficiently protected mitochondrial ISPs from iron-induced injury in vitro. Further In vitro data indicate that both iron chelators and antioxidant drugs likely to reduce iron should be potentially harmful in FRDA. Based on these observations, three FRDA patients with hypertrophic cardiomyopathy were given idebenone orally (5 mg/kg/d). After 4-9 months, quantitative ultrasound evidence of drug efficiency was provided by the reduction of left ventricle hypertrophy in the three patients (mass index: -21, -30, -32%, respectively). These first results provided a basis for a subsequent long-term open trial started April 99 in France. Adults (32) and children (20) with proven FRDA are given idebenone (5 mg/kg/d) for 18 months. Primary criteria of efficiency include heart ultrasound and cerebellar syndrome scoring (WFN ataxia rating scale) each three months. In addition, an audiogram, an estimation of nystagmus and a Steward-Holmes test will be performed each 6 months, and an electromyogram after 1 year of treatment. Results of this open trial will hopefully confirm the preliminary data observed in the three FRDA patients.

Program Nr: 2865 from the 1999 ASHG Annual Meeting

Fabry Disease Bone Marrow Transplantation in a-**Galactosidase A Deficient Mice Reverses Substrate Accumulation, Except in the Kidney.** *C.M. Simonaro[1], R.E. Gordon[2], Y.A. Ioannou[1], R.J. Desnick[1]*. 1) Dept Human Genetics, Mount Sinai Sch Medicine, New York, NY; 2) Dept Pathology, Mount Sinai Sch Medicine, New York, NY.

Fabry disease is an X-linked inborn error of glycosphingolipid metabolism resulting from the deficient activity of the lysosomal exoglycosidase, a-galactosidase A (a-Gal A). The enzyme defect leads to the pathologic accumulation of its major substrate, globotriaosylceramide (GL-3), particularly in blood vessels, heart, and kidney leading to early demise due to vascular occlusions, cardiac disease and renal failure. To investigate the effects of bone marrow transplantation (BMT), 18 newborn a-Gal A deficient mice generated by gene targeting (Wang et al., Am. J. Hum. Genet. 59:A208, 1996) were successfully transplanted with normal adult bone marrow following sublethal irradiation at 400 cGy. Engraftment was monitored by determining a-Gal A and GL-3 levels in peripheral leukocytes and plasma. Twelve of the BMT recipients (6 hemizygous males and 6 homozygous recessive females) remained engrafted up to 38 weeks post-BMT, and several had normal or near normal a-Gal A levels in plasma and leukocytes and activities in the spleen, heart, liver and kidney that were 88, 21, 20 and 8 percent of normal, respectively. The GL-3 concentrations in the plasma and liver decreased to normal, while the pathogenic glycoplipid was reduced by 75% in the spleen and 65% in the heart. Electron microscopy also revealed markedly reduced substrate accumulation in hepatic Kupffer cells, splenic macrophages, smooth muscle and vessels of the heart, and vascular endothelium. However, the GL-3 accumulation in the kidney, a major site of pathology in human Fabry disease, was not depleted by BMT. In particular, the substrate deposition in the podocytes of the glomeruli and the epithelium of the proximal and distal tubules remained unchanged ultrastructurally, and the renal GL-3 concentration was unchanged, if not slightly increased, post-BMT. These results indicate that BMT performed early in life may prevent many pathological and clinical manifestations in affected males, but may have limited, if any, effect on the renal disease.

Program Nr: 2866 from the 1999 ASHG Annual Meeting

Retroviral mediated transfer and expression of b-**hexosaminidase** a-**chain gene in human fibroblasts from B and B1 variant G_{M2}-Gangliosidosis.** *C.A.F. Teixeira[1], M. Sena-Esteves[3], X.O. Breakefield[3], M.C.S Miranda[1,2], M.G. Ribeiro[1,2].* 1) Genetic Neurobiology, I.B.M.C., Porto,Portugal; 2) Unidade de Enzimologia,Instituto de Genetica Medica,Porto,Portugal; 3) Molecular Neurogenetics Unit, Massachusetts General Hospital,Boston,USA.

The variants B and B1 of G_{M2}-Gangliosidosis are recessive fatal lysosomal disorders characterised by the accumulation of ganglioside G_{M2} particularly in the CNS due to b-hexosaminidase A (HexA) deficiency. The variant B1, a common disorder among Portuguese, is distinct from variant B in that the HexA (hetrodimer ab) is synthesized but the a-subunit is inactive. For these disorders no treatment is available and therefore gene therapy could represent a therapeutic approach, specially for variant B1 that is a late-onset disease. We have therefore studied the effectiveness of providing the human a-chain gene via a retroviral vector to HexA-deficient cultured human fibroblasts from B and B1 variant patients as a first step in evaluating the potential of gene therapy for these disorders.

The full-length human a-chain cDNA was subcloned into pBabe retroviral vector under the control of MoMLV LTR element and the recombinant retrovirus used to transfect the packaging cell line y-CRIP. The NIH3T3 cells infected with the recombinant retroviral particles overexpressed the a-chain resulting in the formation of HexS (homodimer aa). With the a-chain specific synthetic substrate 4MU-GlcNAcS, the activity was increased about 10-fold above the non-transduced cell level. The viral stocks which resulted in the highest activity in NIH3T3 cells were subsequently used to transduce cultured human fibroblasts from B and B1 variant patients. In transduced HexA-deficient cells a catalytically active HexA was produced. The enzymatic activity measured with 4MU-GlcNAcS increased about 30-fold above the level of non-transduced HexA-deficient fibroblasts, being within the range of values observed in non-transduced normal cells. Further studies are being undertaken in order to evaluate the value of retroviral-mediated gene transfer in correcting the HexA deficiency in cultured human cells.

Program Nr: 2867 from the 1999 ASHG Annual Meeting

Muscle is a major site for latent adeno-associated virus genomes. *Z. Tezak, E.P. Hoffman.* CNMC CRI III, Washington, DC.

Adeno-associated virus (AAV) is a human parvovirus of no known pathogenicity. Recombinant AAV can infect a large number of cell types, integrate into genomic DNA, and show persistent gene expression. As such, it is becoming an increasing focus for gene delivery in brain, skeletal muscle and liver. About 80% of humans are seropositive for AAV. However, the normal tissue tropism of wild-type AAV has not been well defined; this is of importance both with regards to the nature of this benign integrating DNA virus in humans, and with regards to interpreting gene therapy efforts centered on neuromuscular diseases. The goal of this study was to investigate whether skeletal muscle is a major target organ for wild type AAV. Previously published DNA studies have shown only uterine tissue to consistently and with high frequency test positive for AAV. To analyze whether AAV genomes are present in human muscle, we isolated DNA from 87 skeletal muscle biopsies, and tested for presence of AAV rep sequences by PCR. To ensure that we are not detecting contaminating recombinant AAV sequences, we designed the set of PCR primers recognizing these sequences. We detected wild type AAV DNA in 17% (8/48) of normal human muscle biopsies. Compared to published values for AAV DNA in blood (2/55 or 3.6%) and our own testing of lymphocytes (1/33 or 3%), there is statistically significantly (c^2 is 4.07) more AAV DNA found in normal muscle. We also identified AAV DNA in 10% (4/39) of Duchenne muscular dystrophy patients muscle biopsies. All together, we detected AAV genomes in 14% (12/87) of muscle biopsies tested. We found AAV sequences in biopsies of both genders, ranging in age from 0.5 to 43 years. Automated sequencing showed that AAV sequences were identical to AAV-2 DNA sequence. We subsequently isolated RNA from the same muscle biopsies and could not detect AAV transcripts by PCR. These findings suggest human muscle as a target for latent AAV infection. Our result is of particular interest given that we and others have shown that common helper viruses for AAV (HSV and adenovirus) show exceedingly poor infection of adult muscle. Thus, it appears that latent, integrated AAV genomes are present in tissues that may not be capable of producing viral particles.

Program Nr: 2868 from the 1999 ASHG Annual Meeting

In vivo analysis of photoreceptor degeneration in light-inducecd LACA mice and in vitro ribozyme cleavage of key mRNA targets involved in apoptotic mediated cell death. *In vivoIn vitro*. *G.P. Tuohy[1], P.F. Kenna[1], S.J. Martin[2], P. Humphries[1], G.J. Farrar[1]*. 1) Wellcome Ocular Genetics Unit, Smurfit Institute of Genetics, Trinity College Dublin, Dublin 2, Ireland; 2) Department of Biology, National University of Ireland, Maynooth, Kildare, Ireland.

Retinitis pigmentosa (RP) is a group of genetic degenerative diseases of the retina which afflicts approximately 1 in 4000 of the general population. The genetics of inherited retinopathies display all known mechanisms of inheritance and involve disruptions on nearly every human chromosome. Families exhibiting autosomal dominant, autosomal recessive, X-linked, maternal (mitochondrial) and digenic inheritance are well documented in the literature. Despite the diversity of genetic lesions which give rise to the progressive blindness found in RP patients many forms of the disease are believed to advance through a programme of apoptotic degeneration. An animal model of light-induced apoptotic death in the retina demonstrates a number of the classical markers of apoptosis including morphological changes, TUNEL labelling and DNA laddering. Several studies have demonstrated apoptotic degeneration in mouse models of RP in addition to studies showing activation of caspase 3, a key mediator of apoptotic cell death. Down regulation of caspase activity either by protein inhibition of caspase family members or by employing ribozymes directed against caspase family mRNA may slow or halt the destruction of photoreceptors. This study presents in vitro data of ribozymes directed to cleave caspase 3, 8, 9 and Apaf-1 mRNA targets. Interference with such cell death may in itself represent an effective therapy or may provide a window of opportunity to introduce a gene-based therapy aimed at correcting the primary genetic lesion. In so far as the retina may be representative of fully differentiated neuronal tissue such a strategy may prove beneficial in other diseases involving neurological degeneration.

Program Nr: 2869 from the 1999 ASHG Annual Meeting

The Pathobiology and Treatment of Mucopolysaccharidosis Type VI: Studies in the Cat and Rat Animal Models.

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Mucopolysaccharidosis type VI (MPS VI) is the lysosomal storage disorder due to the deficient activity of arylsulfatase B (ASB) and the resultant accumulation of the glycosaminoglycan, dermatan sulfate. MPS VI is clinically manifested by severe bone and connective tissue deformities, short stature, degenerative joint disease, cardiopulmonary complications, hepatosplenomegaly, and corneal clouding. MPS VI also has been described in cats, rats, and dogs. We evaluated several approaches for the treatment of MPS VI in rats and cats, including hematopoietic stem cell (HSC) gene therapy, bone marrow transplantation (BMT), and/or enzyme replacement therapy (ERT). BMT and ERT in these animal models led to modest improvements in locomotor skills, improved histology in relevant tissues, including the heart, liver and trachea, and some modest reduction of long bone disfigurement. However, the major skeletal abnormalities which are characteristic of MPS disorders were not significantly improved by these approaches. We have also achieved long-term engraftment of retrovirally transduced HSCs in the MPS VI cats and rats without any myeloablative preconditioning, but this approach has been unsuccessful due to low levels of expression in the appropriate target sites, particularly cartilage and bone. Currently, we are evaluating BM stromal cell transplants as a source of progenitor cells for cartilage and bone and developing new forms of the enzyme which may be taken up more efficiently by these cell types. We are also continuing to investigate the pathobiology of these animal models to better understand the disease process and possible avenues of therapeutic intervention. Towards this end we have found widespread and progressive apoptosis in chondroctyes from MPS VI animals, but not age-matched controls.

Program Nr: 2870 from the 1999 ASHG Annual Meeting

Identical mutation in the dysferlin gene can cause either a limb girdle muscular dystrophy type 2B or a Miyoshi myopathy phenotype. *K. Wrogemann[1], E. Nylen[1], T. Weiler[1], R. Singal[1], C.R. Greenberg[1], I.A. Ivanova-Smolenskaya[3], V.S. Sukhorukov[3], R. Bashir[2], K. Bushby[2], S.N. Illarioshkin[3].* 1) University of Manitoba, Winnipeg, MB, Canada; 2) University of Newcastle upon Tyne, Newcastle upon Tyne, UK; 3) Russian Academy of Medical Sciences, Moscow, Russia.

Limb girdle muscular dystrophy Type 2B (LGMD2B) and Miyoshi myopathy (MM) are both caused by mutations of the recently identified dysferlin gene on chromosome 2p13. In a large Russian kindred, eight patients with the proximal LGMD2B phenotype, three with the distal MM phenotype and one preclinical case have been previously described to be homozygous for the same extended haplotype around the dysferlin locus (Brain 119:1895-1909, 1996). This suggested that identical mutations could lead to both phenotypes. We have screened the 55 exons of the dysferlin gene by SSCP/heteroduplex/sequencing analysis and found a dinucleotide change, TG to AT at nucleotide positions 573-574 in exon 3. This leads to a Val67Arg mutation in dysferlin. The mutation segregates as expected for a mutation causing an autosomal recessive disorder, and all patients are homozygous for this mutation. The mutation has not been previously reported in patients with MM or LGMD2B nor has it been seen in 120 control chromosomes. Similar observations have been made in a large Canadian aboriginal kindred with both LGMD2B and MM where patients with both phenotypes are homozygous for a Pro791Arg mutation (Human Molecular Genetics 8:871-877, 1999). Thus, we conclude that diverse dysferlin mutations can cause both LGMD2B and MM and that additional factors, such as modifier gene(s) must contribute to the development of the presenting clinical phenotype.

Program Nr: 2871 from the 1999 ASHG Annual Meeting

Molecular Cloning and Functional Characterization of a Translational Control Protein (TCP) that Binds to Coding Sequences of Acid b**-Glucosidase RNA and Other RNAs.** *Y-H. Xu, C. Busald, G.A. Grabowski.* Div Human Genetics, Children's Hosp Medical Ctr, Cincinnati, OH.

GCase is the enzyme deficient in Gaucher disease; a prototypical inherited metabolic error for enzyme and gene therapies. An 80 kDa cytoplasmic protein, termed TCP80, inhibited GCase mRNA translation in mammalian cells by binding to GCase RNA coding regions. The TCP80 cDNA was cloned from an expression library by screening with the GCase RNA coding region. The cDNA sequence was nearly identical to those for M-phase phosphoprotein (MPP4; 99%) and to an IL-2 enhancer binding protein (NF90; 96%). Expression of the carboxy terminal third of the protein, termed TCP30, showed TCP80 to be an RNA binding protein with specificity for 184 nt of GCase RNA coding sequence. This fragment was located near the 5' end of the GCase RNA. TCP30 diminished the translation inhibition of GCase RNA caused by cytoplasmic TCP80. TCP50, expressed from the NH2 - terminal two-thirds of TCP80, did not bind to GCase RNA nor inhibit its translation. Intact TCP80, heterologously expressed in insect cells, reconstituted in vitro translation inhibition of GCase. Time course analyses showed that TCP80 functions at the initiation phase of GCase mRNA translation, probably by inhibiting its binding to polysomes. These effects could be reconstituted in vivo by co-infection of TCP80 and GCase baculoviruses into Sf9 insect cells that do not constitutively express TCP. Seven additional RNAs were isolated by specific binding to TCP30. In vitro translation of several of these was inhibited by TCP80. These studies show that TCP80 has RNA binding (TCP30) and inhibitory (TCP50) domains that function to modulate translation of several mRNAs with implication for gene-based therapeutic approaches.